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PHYSIOLOGICAL REVIEWS

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THE AMERICAN PHYSIOLOGICAL SOCIETY

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PHYSIOLOGICAL REVIEWS

VOL. 24 JULY, 1944 No. 3

THE ANTICOAGULANTS EFFECTIVE IN VIVO WITH SPECIAL REFERENCE TO HEPARIN AND DICUMAROL[1]

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The successful employment of heparin and dicumarol in the treatment and prevention of thrombosis is largely responsible for the present interest in the anticoagulants. Aside from their utilitarian potentialities, these agents are of special significance to the student of physiology since they furnish important clues concerning the mechanism of the coagulation of the blood. It will be the purpose of this review to present the chemical and physiological action of the principal substances that inhibit the coagulation of blood with only incidental reference to their clinical application. No extensive historical discussion is deemed necessary since the writer summarized much of this material in his recent monograph (77), and Wöhlisch (114), Mason (65), Jorpes (47), Best (6), Prandoni and Wright (73) have reviewed both the older and more recent literature.

THE NORMAL ANTITHROMBIN OF THE BLOOD. It has long been known that plasma or serum will inactivate large amounts of thrombin. There is no evidence that this antithrombic substance in blood is an anticoagulant, since Volkert (107, 108), as well as earlier investigators, have found that changes in its concentration have no demonstrable effect on the coagulation time. The writer (77) has postulated that the affinity of thrombin for fibrinogen is greater than for the plasma antithrombin and as a consequence little thrombin is combined and inactivated by the antithrombin before all the fibrinogen is converted to fibrin.

The writer's observation that the antithrombin resides almost entirely in the albumin fraction has been verified in various laboratories (2, 3, 33, 101, 116), but all attempts to isolate the active constituent have failed. Wöhlisch and Köhler (117) report that the antithrombic potency of serum albumin can be eliminated by extraction with ether or chloroform; and Grüning (39) recently stated that a substance possessing full antithrombic activity can be recovered in the extract. He concluded that the antithrombin is not a protein but a fat or lipoid closely associated with the protein. The fact that the potency of the extract is destroyed at 50°C. makes one hesitate to accept that it is a simple lipoid. Until more concrete information is available, it may be well to continue calling it Albumin X.

Attempts to determine variations in the antithrombin concentration of blood are not new as Astrup and Darling (3) point out, but due to unsatisfactory methods, most of the earlier findings must be regarded as qualitative. Astrup and Darling (3) have developed a quantitative method which consists in incubating

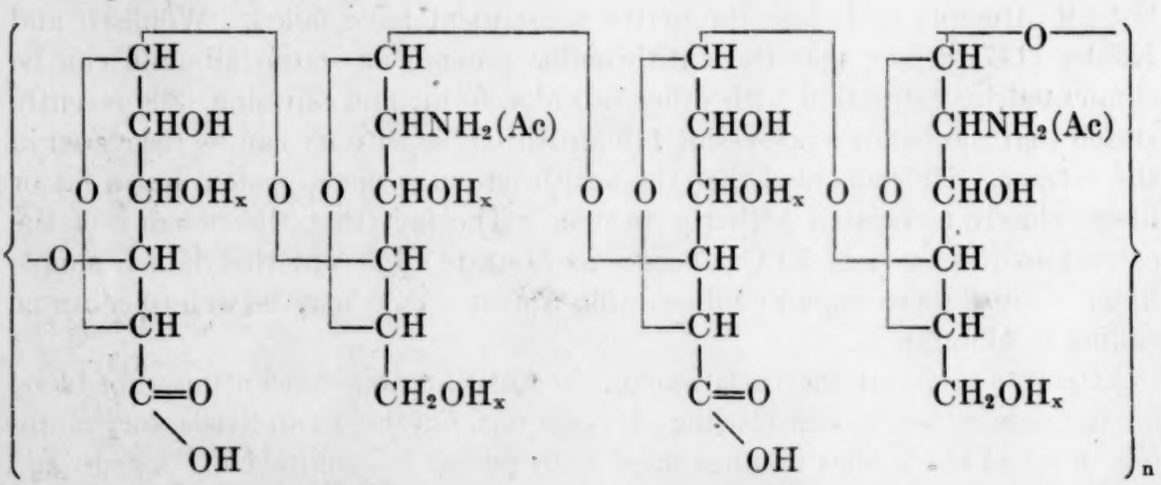
[1] Dicumarol is the name adopted by Link and his associates and is used throughout this review.

1 cc. of thrombin of standard strength with varying amount of serum (0 to 0.2 cc.) at 37°C. for 15 minutes. At the end of this time, the clotting power of the mixture is determined by allowing 0.1 cc. of the mixture to react with 1 cc. of fibrinogen solution. From these data the amount of thrombin consumed can be calculated.

With this method Volkert (107, 108) has found that the normal antithrombin gradually rose to a maximum in about 14 days after injecting soluble proteins such as egg albumin and horse serum into rabbits and dogs. It appears unlikely that this is an immunological response since a similar increase was observed when gelatin, starch and other substances without antigenic properties were injected; whereas no response occurred when corpuscular antigens, such as human erythrocytes, were given intravenously. Curiously, India ink caused a transient drop as did liver injury from chloroform and carbon tetrachloride, while ligation of the common duct increased the antithrombin. Dyckerhoff and Marx (32) also have found an increase of antithrombin in jaundice. Wilson (112), employing a method essentially the same in principle as that of Astrup and Darling but differing in the details of the procedure, has found that the antithrombin content of plasmas of man and the common laboratory animals show no striking differences.

The significance of antithrombin in blood is not known. Wöhlisch (115) voices the same opinion expressed by the writer that it probably serves as a protection against small amounts of thrombin formed intravascularly. One of the perplexing problems is the relation of this normal antithrombin to heparin, which will be presently discussed.

Heparin. *Chemistry.* It is not known whether heparin is a single substance or a group of closely related compounds. Jorpes and Bergström (50) and Charles and Todd (22) agree that heparin contains glucosamine, a uronic acid, which has not been satisfactorily identified, and several sulfuric acid groups in ester linkage. The latter investigators propose the following formula for beef heparin:



The x indicates the probable position of the sulfate groups. It is not known how completely the amino groups of the glucosamines are acetylated. In the formation of salts, such as that of barium, the hydrogens of the sulfate groups rather than of the carboxyl groups are believed to be replaced.

It can be seen that the formula closely resembles mucoitin sulfuric acid. Apparently minor changes in the molecule which may not be detected chemically can markedly change the biological activity. Jaques, Waters and Charles (46) have found that dog heparin has an activity of 240 units per mgm., beef of 100, pork 44, and sheep 23. No chemical differences could be detected. All contained the same percentage of sulfur and nitrogen, and had the same optical rotation. Jorpes (48, 49) also noted the high activity of dog heparin, but he observed variations in the sulfur content. Kuizenga and Spaulding (54) recently recorded that the barium salt of beef lung heparin containing 100 units per mgm. can be separated into two fractions, one of which has a potency of 125 units per mgm.

These results suggest that heparin may not be a single substance with a fixed activity but a mixture of compounds having a mucoitin polysulfuric acid structure, but differing perhaps in the number of sulfuric acid radicals and in other chemical groups.

Action. The behavior of heparin in the blood differs from that observed in a test tube when allowed to react with purified reagents. Heparin by itself is not an anticoagulant. In a mixture of purified fibrinogen and thrombin, it has almost no inhibitory action; and likewise it shows no retarding or blocking effect on the conversion of prothrombin (precipitated from plasma) to thrombin (74). It should also be emphasized that heparin is not neutralized by thromboplastin, a finding that is repeatedly disregarded even in the current literature.

The key to the physiological action of heparin appears to be its strongly acidic property by virtue of which it forms stable salts with many proteins (37). In this reaction the physical and chemical properties of the proteins such as isoelectric points, solubility and electrophoretic patterns are altered. If the protein has enzymatic or other biological activity, one can postulate that heparin may influence, i.e., intensify or inhibit these properties.

Jaques (44) has studied the reaction of heparin on a number of proteins including casein, gelatin, protamine and the dye, toluidine blue. He found in agreement with Fischer's earlier postulate that a stoichiometric relationship exists between heparin and the protein:

$$\text{Protein} + \text{heparin} \rightleftarrows \text{protein-heparin}$$

or

$$\frac{C_{\text{protein}} \times C_{\text{heparin}}}{C_{\text{protein-heparin}}} = K$$

A strongly basic protein such as the protamine, salmine, will form a heparin salt which is little dissociated, while a compound like gelatin when combined with heparin readily undergoes dissociation. Certain factors such as the pH, variations in the anion and cation concentration, and probably the presence of certain lipoids may influence the dissociation of the various protein-heparin complexes.

The anti-thrombic action of heparin. The coagulation of fibrinogen by thrombin is prevented by heparin provided a co-factor is present. This latter agent is found in the plasma, specifically in the albumin fraction. The fact that this co-

factor loses its antithrombic activity in conjunction with heparin when heated to 67°C., suggests that it is a protein similar or identical with a fraction of serum albumin (76). Ziff and Chargaff (119) found that all albumin fractions displayed some activity except the crystalline product. Later these authors (21) observed that serum albumin separates into three fractions electrophoretically and that the fast and middle fractions are active as heparin co-factors, while the slow fraction is not.

One can postulate that heparin unites with a fraction of serum albumin, which for convenience can be designated Albumin X_1 and the resulting complex, Albumin X_1-heparin has the ability to bind thrombin and therefore is a true and powerful antithrombin. Heparin also combines with other proteins, for Chargaff and his associates (21) have obtained evidence that it unites even with plasma globulins. When heparin is added to blood, it will be distributed between the various plasma proteins. Theoretically, qualitative and quantitative variations in the proteins may influence the anticoagulative effectiveness of heparin, but this has received no extensive study either experimentally or clinically. The findings of de Takats (102) that the coagulation time response to injected heparin shows marked variations, may perhaps be a manifestation of plasma protein alterations.

When a strongly basic protein like protamine is added to blood made incoagulable with heparin, the antithrombic activity is immediately lifted (20). Presumably the heparin is removed from Albumin X_1 and combines with protamine, for the dissociation constant of protamine-heparin is smaller than that of the Albumin X_1-heparin complex.

Both the normal antithrombin and the co-factor of heparin are constituents of serum albumin, both are inactivated at 67°C. and both are absent in crystalline albumin. Quick (76) postulated that the two are identical and that when heparin is united with the normal antithrombin its action is so intensified that it becomes a powerful anticoagulant. Seegers and his associates (90, 92) reached a similar conclusion, namely, that thrombin is destroyed by the antithrombin of the plasma and that heparin merely accelerates the speed of the reaction but does not increase the capacity of plasma to inactivate thrombin. They consider heparin to be essentially a catalyst. On the basis of this conclusion Seegers and Smith (91) propose a quantitative method for the assay of antithrombin (i.e., of the co-factor).

Astrup and Darling (2) reject the concept that normal antithrombin and the heparin co-factor are identical. They state that in purifying serum albumin, they obtained fractions that possessed no antithrombic properties but were activated by heparin; and vice versa, fractions that were antithrombic were not further activated by heparin. The actual experimental procedures are not recorded. They mention that the co-factor in ox blood is inactivated by heating at 56°C. for 5 minutes while the antithrombin concentration is not reduced. Further investigation is needed to solve this important problem concerning the relationship of antithrombin to the co-factor of heparin.

The antiprothrombic action of heparin. Howell and Holt (41) in describing

heparin and its action reported experiments which indicated that this agent inhibited the conversion of prothrombin to thrombin. This view was generally accepted until it was shown that prothrombin isolated from plasma is readily converted to thrombin in the presence of heparin (66, 74). Brinkhous and his associates (12) confirmed the observation that heparin alone is not an antiprothrombin, but found that in the presence of serum it completely inhibits the conversion of prothrombin to thrombin. They conclude that heparin acts only in combination with a plasma factor, but from their data, they were unable to say whether the action is antiprothrombic or antithromboplastic. (Since excess thromboplastin does not clot plasma made incoagulable with a minimal amount of heparin, it seems unlikely that heparin plus co-factor constitutes an antithromboplastin.) They offer no information concerning the nature of the co-factor except that it is not dialyzable. Ferguson and Glazko (35) by means of a rather involved type of reasoning conclude that heparin has a direct action and that the first effect is antithromboplastic, i.e., an inhibitory action on the tryptase-like thromboplastic enzyme, while the second effect is on the prothrombin molecule. In addition to this intrinsic action of heparin, a co-factor found in crude plasma albumin markedly potentiates the anti-prothrombic action.

Progress in unravelling this intricate problem is contingent on learning more about the nature of prothrombin and the manner in which it occurs in unaltered blood and in decalcified plasma. Since evidence has been obtained that prothrombin consists of two components combined with calcium, and that removal of the latter ion causes a disruption of the prothrombin complex (78), one can justifiably question whether the inhibitory action of heparin in blood is necessarily the same as in decalcified plasma.

Heparin and platelets. It has been recognized since the time of Bürker (1904) that all agents that inhibit coagulation also prevent the agglutination of platelets. The action of heparin in stopping the clumping of platelets is presumably not directly on these cells but is the result of its anticoagulant property. Baronofsky and Quick (5) found that human blood to which 0.25 mgm. of heparin per cc. was added showed no clumping of platelets or any diminution in their number. The finding of Copley and Robb (25) that heparin causes a decrease of the platelet count both in vitro and in vivo could not be verified.

The starting point of a thrombus is a small mass of agglutinated platelets, and the prevention of this clumping is probably the primary action of heparin in its prophylaxis against thrombosis. The classical work of Best and his co-workers (7) who studied platelet agglutination in the living animal by means of a glass cell (with a transverse scratch on its inner surface) which is connected between the carotid artery and the jugular vein, has done much to clarify the concept of thrombosis and to place therapy on a scientific basis. Interestingly it was found that a dose of heparin which immediately raised the clotting time of a dog to 6 hours, did not prevent the agglutination of platelets (97). To accomplish the latter, large doses (over 300 mgm./kgm. of body weight) were necessary and the effect required 15 to 50 minutes to become manifest. It might be stated that *in vitro* the action of heparin is immediate.

Heparin in peptone and anaphylactic shock. In both types of shock three things occur: a marked diminution of platelets, a liberation of histamine and an outpouring of heparin into the blood stream, which may be sufficiently massive to bring about complete incoagulability. The author in his monograph attempted to correlate these three effects and postulated that heparin served as a defense agent. Unfortunately this speculation was at best premature and has not been borne out by facts. At present there is little evidence to support any relationship between platelets, histamine and heparin. Histamine when injected does not cause any significant decrease in platelets. This observation was reported by Kinselle et al. (52) and confirmed in the writer's laboratory, thus invalidating earlier reports to the contrary. Heparin does not protect rabbits or guinea pigs against anaphylactic shock induced by horse serum. In rabbits the only manifestation following a shocking dose may be a precipitous fall in platelets, without any other gross concomitant signs, and this decrease in platelets is not prevented by excessively large doses of heparin. The depletion of platelets is caused by a marked agglutination immediately after the shocking dose followed by a rapid removal of the platelet clumps. The effect is on the circulating platelets, and is temporary, since full restoration in number occurs in 30 minutes. (Unpublished results.) Since heparin does not appear to inhibit the agglutination of platelets in shock but does prevent platelet clumping due to contact with a foreign or rough surface, one must question whether the two types of platelet aggregation are identical.

Dragstedt and his group (31) have reported that heparin inhibits in vitro the release of histamine from cells to the plasma brought about by trypsin, proteose or a specific antigen. The amount of heparin needed is higher than that required to prevent coagulation. Whether this observation is related to the finding of Landis et al. (56) that the vasoconstricting effect of defibrinated blood can be prevented by heparinizing blood or plasma is not known.

With the actual isolation of heparin from the blood of dogs in anaphylactic shock by Jaques and Water (45) it is now certain that this substance is responsible for the incoagulability of the blood in peptone and anaphylactic shock. As further evidence they demonstrated that the liver after shock contained less heparin than the normal liver, and by histological studies following Wilander's toluidine-blue staining reaction they showed damage to the mast cells, which contain heparin in the form of metachromatic staining granules. The article of Best (6) should be consulted for the work on the mast cells and their relation to heparin. The mechanism and the stimulus responsible for the discharge of heparin from the mast cells into the circulation is still an unsolved problem.

The question whether heparin protects against anaphylactic shock remains unanswered. Recently Macht (64) again reported success in preventing anaphylactic shock with heparin while others including the writer (unpublished results) and Jaques (44) failed to demonstrate such protection. It is possible as Jaques states that it may be difficult to reproduce the necessary condition to demonstrate this inhibitory action of heparin; and he suggests that the action may be explained by assuming that heparin uniting with the sensitizing protein

so changes the antigenic property that it can no longer elicit an anaphylactic shock.

The standardization of heparin. Although no unit of heparin potency has been officially accepted, the crystalline barium salt of the Connaught Laboratories of the University of Toronto can well serve as a standard and the proposal that the unit be defined as 0.01 mgm. of this salt seems desirable. Such a unit is about five times larger than the original Howell unit.

Various methods for assaying heparin have been proposed. Howell's method consists in determining the amount required to keep 1 cc. of cat blood liquid for 24 hours under standardized conditions. This type of procedure has been very useful, but is time-consuming, cumbersome, and subject to various errors.

A second method is based on finding the minimum quantity of material necessary to prevent the coagulation of recalcified oxalated plasma. Foster (38) and Kuizenga and his co-workers (53) have recently described such methods. MacIntosh's (61) method is similar except that he adds an excess of thromboplastin, thus eliminating variability of this factor.

A satisfactory method (77) consists in adding the heparin to oxalated plasma (preferably human) and then titrating with increasing amounts of a standardized thrombin solution, using the clot as the end point. The unit of heparin is expressed in terms of its capacity to neutralize a fixed amount of thrombin. This method has the advantages of simplicity and of yielding reproducible results.

Jaques and Waters (45) have observed that 1.9 mgm. of protamine (salamine) neutralizes 1 mgm. of heparin in dog blood, and from this finding have developed a quantitative procedure for titrating the quantity of heparin in the blood during anaphylactic shock.

MacIntosh (61) has developed a colorimetric method based on the observation that heparin reacts with toluidine blue forming a complex which can be removed with petroleum ether while the unchanged dye remains in solution. The heparin can be calculated from the loss of dye. Copley and Whitney (27) have likewise employed toluidine blue as well as Azure A for determining heparin colorimetrically. They report that 1 unit of heparin (Connaught Laboratory) decolorizes 15 gamma of 100 per cent toluidine blue dye content. This unit equals 25.4 gamma of toluidine blue Nu-3 or 17.2 gamma of Azure A Naz-7. A comparison of the anticoagulant and colorimetric methods shows discrepancies which may be attributed to inherent errors in both types of assay.

The urgent need now is not so much a better method for assaying heparin, but procedures to determine the co-factors of heparin. Since plasmas of various species are apt to vary in their content of these factors, it is to be emphasized that this should be taken into consideration in the interpretation of results. Even differences in thrombin or thrombin-like agents may give rise to misleading results. Thus Rigdon and Haynes (83) found that heparin does not inhibit the clotting agent, coagulase, produced by certain strains of staphylococci. This does not mean necessarily that heparin is ineffective; it may be due to the lack of a suitable co-factor.

Physiological significance. The very fact that the organism possesses a large

store of heparin seems indisputable evidence that it has an important function; nevertheless, its rôle in the body economy has not been found. It is still a moot question whether normal blood contains any free heparin. If it does, the amount is too small to be unequivocably demonstrated. Certain it is that the fluidity of blood can be as convincingly explained by hypotheses that exclude heparin as by those that center about it. The purpose of heparin in anaphylactic shock is, as already stated, quite obscure. To the best of the writer's knowledge, the literature contains no record of any instance in which an abnormal amount of heparin has been found in human blood. The finding of Macht (64) that heparin lowers the toxicity of ouabain and digitalis suggests that it functions as a protective agent, since these substances are reported to increase the coagulability of the blood.

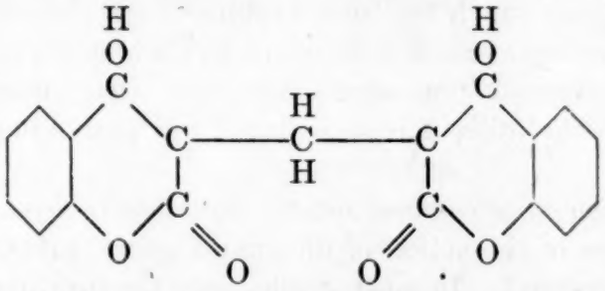
Excess amounts of heparin in the blood are quickly reduced. A portion of the compound is excreted in the urine; as high as 10 to 35 per cent when 200 units per kilogram of body weight are injected into a dog (26). A large amount is not accounted for, but the recent work of Jaques (43) indicates that tissues contain an enzyme, heparinase, which inactivates heparin. The prompt removal or destruction of heparin has necessitated continuous intravenous administration when used clinically but Loewe et al. (60) have found that a prolonged anticoagulant action can be obtained by injecting subcutaneously heparin incorporated in a slowly absorbing medium.

ANTITHROMBOPLASTIN. Antithromboplastins have been proposed from time to time, but usually received but passing notice since the evidence on which the existence of such inhibitory agents was based was usually not convincing. During the past year Tocantins (103, 104) has demonstrated that thromboplastin when incubated with plasma is either destroyed or inactivated. In hemophilia this inactivation is marked, and he believes that this accounts for the delayed coagulation in this disease. He postulates that the thromboplastin is destroyed as fast as it is liberated from the platelets, therefore not enough can accumulate to initiate coagulation. In a later publication Tocantins (105) reports results which indicate that the antithromboplastic activity is directed toward the lipid moiety of thromboplastin, i.e., against cephalin. Interestingly, Russell viper venom is not inactivated by the plasma antithromboplastin. In some respects, protamine when added to plasma acts as the naturally occurring inhibiting agent.

DICUMAROL (3,3′ METHYLENE-BIS-(4 HYDROXYCOUMARIN). The tempo of modern science is dramatically illustrated by the solution of the hemorrhagic disease caused by spoiled sweet clover hay. Two decades after the disease was discovered and described by Schofield (88, 89), Link and his associates (17, 18, 19) not only successfully isolated and identified the compound responsible for the hemorrhagic diathesis, but also described a practical method of synthesis (98) so that the drug immediately became available for both experimental and clinical purposes. The achievement of Link was in no small measure expedited first by the pioneer studies of Roderick (84, 85, 86) who demonstrated that a marked drop of prothrombin occurs; and secondly by the investigations of the writer (75) who reported the first quantitative studies on the effect of toxic

sweet clover hay on the prothrombin level and developed a simple and reliable procedure for determining prothrombin which was adaptable for assaying the hemorrhagic factor in sweet clover hay.

Chemistry. The structure of the toxic principle in spoiled sweet clover is:



3,3′ methylene-bis-(4-hydroxycoumarin)

The compound is a colorless crystalline solid, relatively insoluble in water, acids and in the common immiscible solvents. With strong alkalies it forms soluble salts. The sodium salt solution gels on standing and slowly turns brown probably due to oxidation. A high alkalinity is required (<pH 8) to keep dicumarol in solution, which is somewhat of a disadvantage for intravenous administration. Although the compound is insoluble it is readily absorbed from the gastrointestinal tract and even to some extent when administered rectally (69). The compound is optically inactive. When fused with potassium hydroxide, each molecule yields two molecules of salicyclic acid (98).

The action of dicumarol. The work of Roderick in 1931 (85) and of the writer in 1937 (75) established the fact that the principle of toxic sweet clover hay reduces the prothrombin of the blood and this has been repeatedly verified since dicumarol became available in 1941. Heretofore prothrombin has been considered as a single substance, but recently the writer (78) has presented evidence showing that this agent is composed of two components (A and B) which are linked through calcium. Component A gradually disappears from stored plasma whereas component B is stable. It is component B which decreases in dicumarol poisoning and it is this factor which also appears to be diminished in vitamin K deficiency. This suggests a relationship between dicumarol poisoning and avitaminosis K.

The mechanism whereby dicumarol reduces the prothrombin (or more correctly component B) of the blood is not fully understood, but all evidence points to a depression of the synthesis of prothrombin, i.e., causing an inability to utilize vitamin K rather than to a direct action on prothrombin. This view is expressed by Bingham, Meyer and Pohle (8) and others (1, 42, 72). Witts (113) points out that there is a basic similarity between the structure of 4-methyl 1,4-naphthoquinone and 4-hydroxy coumarin and suggests that dicumarol might act by interfering with the utilization of vitamin K by the liver.

It is not unlikely that dicumarol poisoning is basically similar to carbon monoxide intoxication. Neither agent causes any demonstrable morphological changes. Both impair one vitally important function without disturbing the other activities of their respective organs. Carbon monoxide blocks the oxy-

gen carrying power of hemoglobin, but the other activities of the blood are not directly affected; dicumarol interferes with the synthesis of prothrombin, but does not disturb the other functions of the liver. In both intoxications function is restored when the noxious substance is removed, and removal is hastened by the agent which the toxin excludes from the cell: oxygen in carbon monoxide poisoning and vitamin K in dicumarol intoxication. The most serious sequellae in both types of poisoning come from tissue anoxia; in the one, the anoxia is direct, in the other it is secondary to extensive hemorrhages and subsequent anemia.

Quantitative action of dicumarol on the prothrombin level. Few satisfactory quantitative studies of the action of dicumarol on the prothrombin level of the blood have been reported. In most studies only the prothrombin time is given, but due to the variability of the procedures for determining prothrombin and the differences in activity of the thromboplastin employed, it is not possible to calculate accurately from these data the changes in prothrombin. It is found by nearly all workers that after a single dose, the prothrombin slowly decreases, reaches its lowest point usually in 48 to 96 hours and again becomes normal in about a week. Bollman and Preston (11) state that 10 mgm. of dicumarol per kgm. of body weight given intravenously to dogs caused the prothrombin to fall to about 15 per cent in three days. Overman et al. (70) recorded that a single dose of 2.5 mgm. lowered the prothrombin of a 250 gram rat to 22 per cent of normal in 24 hours. In another paper Overman and his co-workers (72) state that a prothrombin time of 10 minutes has been induced in dogs by a single large dose of dicumarol. Such a result must certainly be the exception rather than the rule.

The writer (78 and unpublished results) has found that the prothrombin decrease follows a fairly uniform pattern in both dogs and rabbits. The prothrombin level falls to approximately:

20 per cent of normal in 24 hours
5 per cent of normal in 48 hours
$1\frac{3}{4}$ per cent of normal in 72 hours
1 per cent of normal in 96 hours

A critical minimum dose is necessary to effect these results, but even greatly increasing the dose above this will not hasten the drop in prothrombin. The pattern remains the same whether the drug is given orally or intravenously. Certain animals are more resistant to dicumarol, a fact which Link (17) found early in his work. The writer has made the interesting observation that chickens are particularly resistant to dicumarol and recover very promptly from its effect.

The findings are best explained by the hypothesis that dicumarol inhibits the enzymatic mechanism which produces prothrombin. The decrease observed actually represents the body's consumption or destruction of prothrombin. As the enzymatic system is freed of the toxin, it gradually regains its capacity to synthesize prothrombin. The beneficial action of vitamin K, which has been reported, can be explained by assuming either that it can replace the toxin,

or that the synthesizing cells remaining unaffected can manufacture more prothrombin when an excess supply of vitamin K is available.

Quantitative determination of prothrombin. Since much of the work on dicumarol depends on the determination of prothrombin, a critical discussion of the one-stage method is indicated. Unfortunately the two-stage method of Smith has not been used extensively so that comparisons of results obtained by the two methods cannot be made. McGinty and his associates (62) report that after giving a dog two doses of dicumarol (14 mgm. per kgm. of body weight) the prothrombin determined by the two-stage method dropped to below 20 per cent of normal in 48 hours. This and their other results agree in general with the writer's data obtained with the one-stage method.

The British investigators have been particularly critical of the one-stage method and have condemned it in no uncertain terms. Macfarlane (63) writes: "It is clear that Quick's test has broken down completely where the coumarin compound is concerned." Witts (113) is equally outspoken and he proposes changing the name, "prothrombin time" to "accelerated clotting time". Most of the criticism centers about the occasional finding that the coagulation time was shorter than the prothrombin time. Such anomalous results have been reported by Bingham et al. (8) and by Davidson and MacDonald (29). The latter recorded that on one occasion, the prothrombin time was over 1 hour while the coagulation time was 17½ minutes and the clotting time of recalcified plasma 9 minutes. In contrast to such results Townsend and Mills (106) record a case with a prothrombin time of 420 seconds and a corresponding coagulation time of 39 minutes; and later of 566 seconds and 60 minutes respectively. The writer has in his unpublished files many similar results, and has never observed a coagulation time which was shorter than the prothrombin time. Butsch and Stewart (13) have reported a series of cases with both prothrombin and coagulation times and in every instance the latter was many times more prolonged than the former.

It should be obvious that discordant results can come from faulty technique equally as readily as from basic defects of the procedure. Accurate timing of clot formation becomes difficult when the prothrombin falls below 1 per cent of normal. Poor thromboplastin is undoubtedly the cause of much inaccuracy. The writer has explicitly described the method for preparing a highly active thromboplastin with a remarkable constancy of potency. There is no longer any valid excuse for reporting prothrombin data based on thromboplastin which does not yield a prothrombin time of 12½ seconds for human plasma and 6 seconds for dog or rabbit plasma. Russell viper venom which is recommended by Hobson and Witts (40) has not yet been satisfactorily standardized for the quantitative estimation of prothrombin. Particularly confusing is the fact that the activity of the venom is markedly potentiated by lecithin. It is difficult to determine from the literature whether the venom should be used with or without lecithin in the test and how the results should be interpreted.

There is no evidence that Link's modification (17) of the original one-stage method, which is essentially running the prothrombin time on plasma diluted

with 7 volumes of saline solution is more accurate. The claim is made that it is more sensitive to small changes of prothrombin, but since the range in which the greatest accuracy is usually desired especially for clinical purposes is below 20 per cent of normal, increasing the prothrombin time by dilution offers no advantage, and is actually undesirable for low prothrombin values.

The best evidence for the reliability of the one-stage method is the finding that when a dicumarinized animal is given a transfusion of normal blood, the calculated expected rise of prothrombin and the actual increase found agree remarkably well. This experiment is routinely demonstrated to our students.

Vitamin K and dicumarol. The studies of the action of vitamin K on the hypoprothrombinemia induced by dicumarol are difficult to evaluate. The writer in 1937 (75) believed he had evidence that alfalfa in the diet of rabbits counteracted the action of toxic sweet clover, but Smith (96) failed to find that this material had any protective action. Campbell et al. (17) likewise found that 2-methyl 1,4-naphthoquinone or natural vitamin K did not prevent the reduction of prothrombin by toxic sweet clover. Bingham and Meyer with Pohle (8) and later with Axelrod (67) found that the hypoprothrombinemia was not influenced by synthetic vitamin K. Allen, Barker and Waugh (1) as well as Wright and Prandoni (118) and Lehmann (57) reported similar observations. Bollman and Preston (11) noted that dogs when fasted were more susceptible to dicumarol, but this disappeared when vitamin K was administered. Both Lehmann (58) and Townsend and Mills (106) have reported satisfactory clinical responses from transfusions plus vitamin K in severe hemorrhagic conditions produced by dicumarol, but the prothrombin level was not followed accurately enough to allow interpretation of these results.

King and his associates (51) presented findings which suggested some antagonism of alfalfa to dicumarol. Overman and his co-workers (70) reported that rats on a diet free of vitamin K showed the maximum susceptibility to dicumarol. All forms of vitamin K, natural as well as synthetic, counteracted the hypoprothrombinemia, and the authors express the opinion that the main effect is a stimulation of the recovery process. A few months later these workers (71) published further data substantiating the antagonistic action of alfalfa on the effect of single small doses of dicumarol. They made the interesting observation that menadione and *l* ascorbic acids when fed at high levels successfully counteracted small single doses (2.5 to 5 mgm.) of dicumarol in rabbits. Menadione by itself was effective, and in some rabbits even *l* ascorbic acid alone was antagonistic to the toxic sweet clover agent. In all these experiments a single small dose of dicumarol was administered. Shapiro and his associates (93) using the same technique have verified the above finding. Davidson and MacDonald (29) found no antidotal action of synthetic vitamin K to dicumarol, but later (30) reported that vitamin K_1 oxide in large doses (as high as 250 mgm.) prevented or reversed the hypoprothrombinemia produced by dicumarol in humans, but also in these experiments only a single dose of dicumarol was given. It seems fairly well established that by rather drastic methods, some antagonism of vitamin K to the action of dicumarol on prothrombin can be demonstrated,

but other factors probably play a part. More information and study are needed before definite conclusions can be drawn.

Transfusion and prothrombin response. The writer (75) in his early studies recorded a sharp but transient rise of prothrombin in dicumarinized rabbits after a transfusion of fresh blood. This observation has been repeatedly confirmed. In view of the recent finding that prothrombin is composed of two components and that factor A disappears when plasma is stored while factor B is diminished in dicumarol poisoning, the use of "banked" plasma should be equally as effective as fresh blood. Cahan (16) in one case has obtained an effective response from citrated banked blood whereas Wright and Prandoni (118) found it ineffective in one case. Obviously more study is required before any conclusions can be drawn especially since it has even been reported that transfusions with fresh blood were ineffective in some patients (111). McGinty and co-workers (62) have obtained promising results with the intravenous injection of purified prothrombin for correcting the depletion of this agent from dicumarol poisoning.

Effect of dicumarol on the liver. Other than depressing the prothrombin, which is synthesized in the liver, dicumarol appears to exert no other deleterious effect on this organ. Animals can be repeatedly subjected to courses of dicumarol poisoning without sustaining liver injury as Link and others have repeatedly observed. On autopsy such animals are found to have livers which are entirely normal (8, 11, 15). Liver function tests likewise show no impairment of the organ (1, 14, 29, 118). Even after administering dicumarol continuously for 92 days to a patient, no detectable changes in liver function were observed by Bingham and his co-workers (9).

According to Rose, Harris and Chen (87) central necrosis of the liver is fairly common in rats after subjecting them to repeated doses of dicumarol. Similar lesions were also occasionally observed in mice and rabbits. Richards and Cortell (81) also noted fatty infiltration of the liver and scattered areas of necrosis. In guinea pigs the livers were not unlike those observed in vitamin C deficiency. Since many of these animals had an advanced anemia, it seems rather probable that the liver damage seen might have been due to anoxia rather than to the direct result of the drug. It is a characteristic finding clinically that patients rarely show any untoward symptoms except hemorrhage (4). In animals that receive lethal doses (40 mgm. or more per kgm. of body weight given intravenously) a dark and congested liver was found (109) but such doses are never given clinically.

Factors influencing the action of dicumarol. It has already been stated that certain animals are more resistant to dicumarol than others. Whether normal men differ in their susceptibility is not known, since all reported studies have been made on patients. Wright and Prandoni (118) found that age and sex had no influence, and that no significant correlation existed between malnutrition and the frequency of toxic reactions. Butsch and Stewart (14), however, record that debilitated and cachectic patients had a more prolonged and a greater elevation of the prothrombin time than did the average patient.

To what extent impaired liver function influences the toxicity of dicumarol

is not definitely known. Shapiro and his associates (94) noted that patients with cirrhosis showed a decrease of prothrombin when given 50 mgm. of dicumarol, whereas patients without liver dysfunction showed no effect from such a small dose. Likewise rats given a hepatotoxin such as carbon tetrachloride were found by Richards and Steggerda (82) to have an increased response to dicumarol. In bilaterally nephrectomized rats, the prothrombin after a single dose of dicumarol continued to drop until death (82) whereas in a normal rat the prothrombin begins to rise after the second day. This experiment illustrates the importance of the kidney in eliminating the toxic principle from the body. After anesthetic doses of chloroform, pentobarbital and pentothal, the prothrombin response to dicumarol is not materially different from that of unanesthetized rats (94).

Most interesting are the findings of Field et al. (36) that lactating rats are unusually resistant to the action of dicumarol and that this protective period lasts throughout lactation even when abnormally prolonged. Perhaps the observation that cows fed toxic sweet clover hay may survive parturition, whereas the newborn calf dies of hemorrhage may be accounted for on the basis of Field's findings. Fever experimentally produced in rats caused a heightened susceptibility to dicumarol according to Richards (80). The clinical implications of these findings are obvious. It would be interesting to see whether a high intake of ascorbic acid can counteract the animal's increased sensitivity to the drug during fever. Since the hypoprothrombinemia due to dicumarol is increased in scurvy both in extent and duration (101a) any condition causing an abnormal consumption of vitamin C is apt to exaggerate the effect of dicumarol.

Dicumarol and capillary fragility. Bingham, Meyer and Pohle (8) stressed that dicumarol causes widespread vascular dilatation of the viscera of dogs especially after massive doses. Bollman and Preston (11) report similar findings after large doses (100 mgm. per kgm. of body weight), and Dale and Jaques (28) likewise state that they have confirmed the result of Bingham et al. Cahan (16) has described a case of purpura following dicumarol (100 mgm. daily for 32 days). A bleeding time of 13 minutes was found, but clot retraction was normal, indicating that the platelets were not involved. Usually the bleeding time is not prolonged even when the coagulation time is greatly delayed (1, 29, 55). Wright and Prandoni (118) emphasize that they could detect no increase in capillary fragility even in frank hemorrhagic cases. Why there should be such a discrepancy in results is difficult to understand, but curiously even the reports on as simple a procedure as the sedimentation rate are confusing. Allen et al. (1) state that the sedimentation rate is routinely increased while Wright and Prandoni (118) find no such change.

Dicumarol in the prevention of thrombosis. The primary objective of much of the work, on dicumarol is its utilization for the prevention of thrombosis. The writer in his studies of 1936 observed that heart puncture in rabbits whose prothrombin was reduced to less than 10 per cent invariably caused death due to hemopericardium. This indicated a loss of the organism's ability to form a thrombus to seal the mural puncture. Specific experimental proof that throm-

bus formation is inhibited by dicumarol was furnished by Dale and Jaques (28) who used the technique already referred to under the section of heparin (7). Richards and Cortell (81) likewise found that dicumarol reduced the tendency of thrombosis following the injection of ethanolamine oleate into the peripheral veins of dogs. Bollman and Preston (11) have also noted the ability of dicumarol to prevent intravascular clotting and have utilized this finding for preparing animals to be used in physiological experiments that require non-coagulable blood.

Essentially the inhibition of platelet agglutination is the primary step in the prevention of thrombosis. When the prothrombin is reduced to a low level, and the coagulation is greatly delayed, platelet clumping no longer occurs as Dale and Jaques (28) have convincingly demonstrated. Baronofsky and Quick (5) found that no agglutination in vitro of rabbit blood occurred after the coagulation time was prolonged by dicumarol, and that an accurate platelet count could be made without using any additional anticoagulant. Obviously one of the essential needs for the rational use of dicumarol clinically is an exact study correlating the coagulation time and the level of prothrombin with the inhibition of platelet agglutination.

Salicylates as the cause of hypoprothrombinemia. Link and his associates (59) noted that rats on a vitamin K-poor diet when given salicylates either orally or intravenously developed a hypoprothrombinemia which could be completely prevented or counteracted by giving the animal synthetic vitamin K. Dogs and rabbits were found to be much more resistant to the action of salicylates, but after liver injury with chloroform a reduction of prothrombin could be effected with this drug. Rapoport and his associates (79) independently also discovered this action of salicylates and showed that not only animals, but also children could develop a low prothrombin due to large doses of sodium salicylate or acetylsalicylic acid. Meyer and Howard (68) observed that relatively small doses of salicylates produced demonstrable hypoprothrombinemia, and in agreement with Link found that vitamin K prevented this effect. Further confirmatory evidence concerning the decrease of prothrombin from salicylates has come from Shapiro and his group (95). They record the interesting finding that dicumarol and salicylates complement each other's action. In cirrhosis the action of salicylates is more pronounced. It seems that the consensus of opinion of all these workers is that the action of the salicylates is essentially the same as that of dicumarol but is less powerful.

Little work has been done on the correlation of structure and the physiological action of drugs depressing prothrombin activity. Fantl (34) has found that 3 methyl, 4-hydroxy coumarin and methylene-bis-(-dimethyldihydroxyresorcinol) is inactive, but that the homolog of dicumarol 3,3′ ethylidene bis (4-hydroxy-coumarin) has the property of producing hypoprothrombinemia which is however less severe and shorter in duration.

The action of sulfaguanidine in reducing the prothrombin level of the blood presents a number of important aspects (10). Vitamin K restores the prothrombin concentration but has no effect in reversing the growth inhibition; *p*-amino-

benzoic acid as well as liver extract restores both growth and the prothrombin level. The most obvious explanation is that sulfaguanidine inhibits bacterial activity on which the rat depends for at least part of its vitamin K requirement. Since *p*-aminobenzoic acid is also effective when given parenterally, the simple explanation does not appear adequate as pointed out by the authors. Collins and Hoffmann (23) report a hemorrhagic diathesis in a case of sprue in which succinylsulfathiazol produced a severe hypoprothrombinemia. Recently Wakim, and his associates (110) demonstrated that cecectomized rats on a vitamin K-free diet when given succinylsulfathiazol invariably developed a severe hypoprothrombinemia, which in many terminated with hemorrhage. Normal rats on the same diet and with the same quantity of the drug only occasionally showed a reduction of prothrombin. Obviously the sulfonamides *per se* have no demonstrable action on prothrombin, but the members of the group which specifically act against intestinal infection can cause a lowered prothrombin, probably by suppressing bacterial activity and thereby reducing the synthesis of vitamin K. The prothrombinopenia is therefore due to lack of this vitamin.

SUMMARY

The normal antithrombin of the blood is closely associated with the albumin fraction. It does not inhibit or retard coagulation, but merely inactivates thrombin. Quantitative methods have been developed for its determination, but the significance of its variation in the blood remains obscure.

Heparin, the natural physiological anticoagulant, is a compound closely related to mucoitin polysulfuric acid. Due to its strongly acidic character, it forms complexes with various proteins and other biological compounds. Heparin *per se* is not an antithrombin, but with a co-factor present in serum albumin forms a strong thrombin-inactivating complex. Heparin in inhibiting the conversion of prothrombin to thrombin likewise requires a co-factor which is present in the plasma. Heparin prevents the agglutination of platelets, probably by virtue of its anticoagulant action. The function of heparin in the body has not been determined. It is liberated during anaphylactic and peptone shock, but the mechanism whereby this is brought about is still obscure and the purpose of this physiological response has not been ascertained.

Antithromboplastin has been demonstrated in the blood, and evidence has been found that it is abnormally increased in hemophilic blood.

Dicumarol is the toxic principle isolated from spoiled sweet clover hay. It is a coumarin derivative which had not hitherto been known to occur in plants. When dicumarol is administered orally or intravenously to man or animals, it causes a gradual but severe decrease of the prothrombin of the blood (or more accurately of component B of prothrombin). Several days are required for the production of its full effect, and recovery is equally slow. Evidence is accumulating which suggests that vitamin K has some antagonistic action against dicumarol. The hypoprothrombinemia produced by the drug can be temporarily alleviated by transfusion. Dicumarol appears to have no toxic action other than depressing the prothrombin except in excessive dosage. It is probable that some

of the pathological findings in fatal cases of dicumarol poisoning can be attributed to tissue anoxia due to the severe anemia after excessive hemorrhage.

Salicylates especially when given to animals on a low vitamin K diet depress the prothrombin of the blood but to a much smaller degree than dicumarol. Sulfaguanidine and succinylsulfathiazol also cause a hypoprothrombinemia, but the action appears to be due to a depression of the synthesis of vitamin K by the bacteria of the intestines and not to a direct action on the synthesis of prothrombin.

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THE HYPERPNEA OF MUSCULAR EXERCISE

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The history of respiratory physiology for the last 60 years has been a succession of attempts to explain all deviations from normal respiration by one theory, whether it be regulation by oxygen content of the arterial blood (133), by alveolar or arterial CO_2 tension (71), by arterial blood pH (161), (79) or by acidity of the interior of the cells of the respiratory center itself (61). But no one theory yet offered can account for all or even a large part of the hyperpnea of muscular exercise, and each of these theories has failed to gain general acceptance because of its inability to explain this most common and most powerful of all respiratory adjustments. Of one thing, however, there can now be little doubt, namely, that the action of known chemical substances directly upon the respiratory center can no longer be regarded as the most important feature in the control of respiration (141). This seemingly drastic conclusion has resulted from several factors, one of which was the brilliant discovery of the pressure receptors in the carotid sinus and aortic arch and of the chemoreceptors in the carotid and aortic bodies (83). This led to the realization that since reflexes had been demonstrated clearly to assume a share of respiratory control formerly accredited to the action of chemical substances upon the respiratory center, then other anomalies of respiration previously classified as "exceptions to the rule" might also be due to reflex factors. As a result of the new outlook, sufficient experimental work has accumulated in the past decade to warrant an even more revolutionary statement: Control of respiration by CO_2 appears to be limited to man at rest or performing mild exercise (and to such laboratory experiments as voluntary hyperventilation and inhalation of CO_2 mixtures); under all other conditions, increased ventilation is caused by factors other than CO_2, and while these may be varied, usually they are of reflex origin. An inquiry into the causes of the hyperpnea of muscular exercise will document this statement for this hyperpnea entails the interaction of practically all the factors known to influence respiration, both central and reflex.

This review will attempt to evaluate the importance of each of these factors and to estimate the contribution of each to the total hyperpnea of exertion.

1. THE EFFECT UPON RESPIRATION OF METABOLITES FORMED DURING EXERCISE. A. *The action of CO_2.* "If ever there was a conviction firmly entrenched in physiology, it was the monopoly of the chemical control of breathing by the respiratory center. Based as it was upon circumstantial evidence, it proved to be one of physiology's outstanding creeds. . . . Central stimulation was simply taken for granted. Indirect evidence was accepted as direct proof. Statements going unchallenged were eventually accepted as facts." (62).

This creed was firmly established by the brilliant work of Haldane (72) who demonstrated beyond doubt that CO_2 is a powerful respiratory stimulant and that the normal respiratory center is exquisitely sensitive to changes in alveolar

or arterial CO_2 tension. This has been amply confirmed by many investigators: Campbell, Douglas and Hobson (31) showed that a 2 mm. increase in alveolar CO_2 tension led to a ventilation increase of 10 liters per min.; Douglas and Havard (54) demonstrated an increased respiration of 32 liters per minute with an increase of only 5.6 mm. in alveolar CO_2 tension; Barcroft and Margaria (7) obtained a maximum ventilation of 71 liters per minute upon inhalation of 7.5 per cent CO_2.

It was only logical to consider that the hyperpnea of exercise might be due to the increased CO_2 produced by the actively working muscles with consequent stimulation of the respiratory center. Indeed Haldane found an increase in alveolar pCO_2 in mild exertion and this has been confirmed by Hough (90). However an overwhelming amount of evidence now exists to indicate that alveolar or arterial pCO_2 does not play an important rôle. For example, Barcroft and Margaria (7) showed that while severe muscular exercise increased ventilation to as much as 115 liters per minute, the maximum hyperpnea obtainable with inhaled CO_2 in the same subject was only 71 liters per minute; this has been confirmed by Nielsen (119) and indicates that some factor or factors in addition to CO_2 must operate to produce a ventilation almost double that possible with CO_2.

Furthermore quite early it became manifest that there was no positive correlation between alveolar or arterial pCO_2 and ventilation during exercise. Hough (90) showed that while alveolar pCO_2 was elevated during mild exercise, it usually returned to normal with more severe exercise. This has been confirmed by Harrison and co-workers (75) and by Krogh and Lindhard (99). In severe muscular exertion, the alveolar and arterial pCO_2 are invariably reduced below normal by 1 to 13.5 mm. (90, 103, 50, 25, 45, 14, 41). It must be concluded *a*, that under no circumstances can the maximal hyperpnea of exertion be due to CO_2 alone, and *b*, since the pCO_2 is rarely above normal in moderate exercise and never above normal in strenuous exertion, the rôle of CO_2 must be a minor one limited to mild muscular movements. However, the marked increase in metabolic CO_2 does prevent too great a fall in arterial pCO_2 and thus provides a favorable environment for the optimal functioning of the respiratory center; this appears to be a matter of great importance and will be alluded to later.

B. *The action of lactic acid.* Noting the lack of correlation between ventilation and alveolar pCO_2, Winterstein (161) and Hasselbalch (79) proposed a modification to Haldane's original concept to the effect that arterial pH rather than pCO_2 was the effective regulator of respiration. Campbell et al. (30, 31) calculated that the respiratory center was so exquisitely sensitive to arterial pH that it responded to changes beyond the limits of existing methods for the detection of pH; a 100 per cent increase in respiration volume was postulated for each decrease in pH of 0.013 unit. Despite the fact that respiration was shown to increase in the face of decreased arterial acidity (61) as in anoxemia (27), hemorrhage (5), anemia (13), fever (70, 68), cardiac decompensation (58), intravenous bicarbonate injection (36), and despite Scott's demonstration of a very low sensi-

tivity of the respiratory center pH changes (144), the Winterstein-Hasselbalch hypothesis was accepted by most physiologists. It appeared to offer the best explanation for the hyperpnea of muscular exercise for, following Ryffel's discovery of the formation of lactic acid by contracting muscles and its frequent spill-over into the blood stream (134), it was logical to assume that the increased acidity of the blood could provide the stimulus to the center when the arterial pCO_2 had fallen below normal. There is ample confirmation of the fact that high levels of blood lactate[1] may be found following severe exercise (85) and that blood pH may be reduced markedly (103, 155, 16). Lactic acid is formed when there is a disproportion between the intensity of the muscular work and the oxygen supply to the muscles; it is formed therefore not only during severe muscular exercise in a normal individual but also when milder muscular work is performed during arterial occlusion or during systemic anoxia. Static effort, in which the circulation to the active muscles may not increase in proportion to the effort involved, probably represents another example of anaerobic work, though this is disputed by Rein and Talbott (131).

However, it has been demonstrated by a number of investigators (42, 111, 112, 118, 40, 123, 25, 73) that blood lactate does not rise during mild exercise as believed formerly (85, 106), and does not necessarily rise with moderate exercise though oxygen consumption or respiration is increased 3–4 fold. An initial increase in blood lactate of exercising athletes was found by Bang (6); this was not sustained and returned to a normal level though the work and the hyperpnea continued unabated. The severity and duration of exercise required for spill-over of lactic acid into the blood varies with the individual (probably depending in part upon training) but at any rate respiration may be increased markedly during exercise with no significant deviations from the normal arterial pH or pCO_2 (Harrison et al., 75; Nielsen, 120). Bock et al. (25) found that De Mar (the famous marathon runner) on one occasion had a ventilation of 90.7 liters per minute with only an insignificant change in lactate and a decrease in arterial pCO_2. In addition there is considerable evidence that, when lactate does increase in the blood and pH decreases, there is little or no correlation between minute volume of respiration and arterial pH. It has been shown that immediately after severe exercise, blood lactate may be increasing (85) and blood pH decreasing (15) while respiration is decreasing abruptly. Barr (16) measured arterial pH and respiratory minute volume during and immediately after severe exercise. In one instance pH was 7.13 during exercise when ventilation was 60 liters per minute, while after exercise the arterial blood was even more acid (pH 7.09) though ventilation had fallen to 31.7 liters per minute In a second subject, the arterial pH during and after exercise was practically the same (7.20, 7.21) while the corresponding volumes of respiration were 51 and 14.6 liters.

[1] High blood lactate does not necessarily indicate a decrease in blood pH since lactic acid reacts with $BHCO_3$ in the blood to form B lactate with the liberation of CO_2. (Blood proteins also release some base thus aiding in buffering the lactic acid (155).) On the other hand, a decreased blood pH due to exercise is usually associated with a high level of blood lactate, though unidentified acids could be present.

Barman et al. (9) have presented curves for blood lactate and ventilation in the post exercise period showing poor correlation between the two, lactate remaining high at a time when respiration had returned toward normal. Bock et al. (25) observed in two exercising subjects who had approximately the same ventilation (45 and 48 liters per minute) that the decreases in pH were markedly different (0.02 and 0.15 respectively).

It seems to be well established therefore that the hyperpnea of muscular exercise is not necessarily due to a decrease in arterial pH. It is a much more difficult matter to assess the proportion of the total hyperpnea which may be attributed to changes in arterial blood acidity when the latter occur. This question can be approached best by observing the degree of hyperpnea that occurs when similar changes in pH occur in the absence of muscular activity. Unfortunately very few measurements have been made of respiratory minute volume in clinical conditions associated with a fall in pH, such as diabetic or nephritic acidosis, and those results that have been recorded are not consistent; cases of equal severity have been reported with pulmonary ventilations as low as 10.6 (113) and 11.4 (98), and as high as 55 (114) liters per min. This suggests that many complicating factors, such as fever, heart failure, pneumonia or peripheral circulatory failure, may be present in very ill patients and so prevent a clean cut analysis. Consequently investigators have produced a fall in arterial pH experimentally by the ingestion of ammonium chloride in resting healthy men and have come to the conclusion that the respiratory mechanism is relatively insensitive to pH changes. Harrison et al. found an increase of only 0.1 liter per minute associated with a pH drop of 0.07, Nielsen (120) found a rise of 0.7 liter per minute associated with a fall in pH of 0.08 unit, and Dennig et al. (49) observed an increase of 3.1 liters per minute with a drop of 0.2 pH unit.

Results such as these have led some to doubt that acidity, either of the arterial blood or of the cells of the respiratory center, is the characteristic stimulus to respiration as suggested by Gesell (61). There is now available acceptable evidence to show that the true stimulus is not acidity but CO_2 as originally proposed by Haldane and Priestley in 1905 (71). While it was thought for many years that CO_2 acted only by reason of its acidity (its special virtue being the rapidity with which it diffuses through tissues (92)) it now seems more likely that it acts specifically. The evidence is as follows: Hooker, Wilson and Connett (88) perfused the medullary centers of dogs with two types of blood, one made acid by the addition of 5 per cent CO_2 and the other made equally acid by the addition of HCl; marked increases in respiratory volume, as much as 3069 per cent, following perfusion of the CO_2-blood while the maximum increase following perfusion of the HCl-blood of the same pH was 128 per cent. Since it may be objected that a certain level of CO_2 is necessary for the initiation of respiratory movements, and that the pCO_2 must have been very low in the HCl-blood, these experiments cannot be regarded as conclusive. However more recently evidence of a different type but leading to the same conclusion has been obtained. Nielsen (120) working with a normal human subject obtained an increase in respiration of 10 liters per minute by CO_2 inhalation with a fall in arterial pH of 0.03

unit, while respiration increased only 0.7 liter per minute following long-continued ammonium chloride ingestion with a pH drop of more than twice this magnitude (0.08 unit). In this connection, the experiments of Comroe are of interest; he injected solutions directly into the medulla of cats with a microtechnique utilizing the Horsley Clarke apparatus and demonstrated that the cells of the respiratory center frequently could be stimulated by bicarbonate buffers but rarely by injections of lactic or hydrochloric acids (38).

While these experiments indicate that CO_2 and not acidity is the natural stimulus to the respiratory center, they obviously do not mean that increased acidity does not stimulate respiration at all. Regardless of the controversial question as to whether acidity constitutes a strong, moderate or weak stimulus at the center, it can stimulate respiration powerfully through reflexes aroused in the carotid bodies (see p. 327). A compromise view, which is in agreement with the few observations reported, would be that small changes in acidity stimulate only weakly while large changes (0.3 to 0.4 unit) may produce powerful respiratory stimulation. Many more well controlled observations of respiratory minute volume and arterial pH (over the whole range compatible with life) should be obtained on otherwise normal subjects in order to settle this point. In view of the deeply rooted conviction among physiologists that acidity and respiration are closely correlated, it is amazing to see how few measurements have actually been made.

C. *Effect of other metabolites.* There remains the possibility that products of muscle metabolism other than CO_2 or lactic acid may be responsible for the hyperpnea of muscular exercise, such as the unidentified chemical stimulant postulated by Geppert and Zuntz (60) or the "respiratory X" of Y. Henderson (67). It is improbable that lactate has any specific functions as a respiratory stimulant (38). There is some doubt as to whether potassium normally enters the blood from exercising muscles (96) but it definitely diffuses into the venous blood when the arterial circulation is occluded (2); it could stimulate respiration centrally or reflexly through the carotid and aortic bodies but it is apt to depress in slightly higher concentrations (57, 38, 89, 160). Phosphates, formerly thought to increase in the blood during muscular exercise (123), probably do not (59). Other products of muscle metabolism have not been tested for their possible effects upon the respiratory center.

2. Reflexes arising from the exercising limbs. Two factors delayed recognition of the part played by reflexes from the exercising limbs: one was complete satisfaction with the Haldane concept and the other was the fact that Geppert and Zuntz (60) in 1888 felt that they had excluded satisfactorily any reflex factor when they stimulated the lower limbs electrically in dogs and obtained respiratory stimulation after transection of the spinal cord[2]. Consequently when Harrison et al. (74, 75, 76) described reflexes from moving limbs, their

[2] It is possible that Geppert and Zuntz tetanized the limb muscles; if such were the case they were dealing not with rhythmic movement of the limbs, but rather with static effort which tends (by its limitation of blood flow through the muscles) to produce anaerobic contraction and lactic acid formation.

work went practically unnoticed. In five dogs with the leg amputated at the hip (the femur fixed in a vise) leaving only the femoral vessels and sciatic nerve intact, passive movements at the rate of 300 per minute led to an immediate augmentation of respiration. To be sure the results were not striking, the increases in minute volume of respiration in these experiments being 5.6, 5.9, 3.5, 12 and 32.6 per cent when the vessels were open and 3.3, 6.0, 1.0, 12.0 and 21 per cent when the vessels were occluded; after section of the sciatic nerves, no increase in ventilation was obtained. However, this work has been confirmed and extended recently by Comroe and Schmidt (37). In their experiments, passive movements (200/min.) of disarticulated legs in anesthetized dogs increased respiration from 22 to 125 per cent (average, 52 per cent). In 86 tests in 50 normal human subjects, passive movements of one leg at the knee at the rate of 100 per minute led to an average increase in pulmonary ventilation of 40 per cent. The hyperpnea could be abolished by nerve section or by local anesthesia about the knee in dogs and by spinal anesthesia in man. It did not appear to arise from the stretch of muscle or muscle tendons and, by exclusion, the site of origin was assigned to joint receptors.

Though it appears certain that some type of proprioreceptors can set up reflexes from an exercised limb, more precise information is needed as to the contribution of these reflexes to the total hyperpnea of exertion. Comroe and Schmidt felt that these reflexes were not powerful enough to account for more than a small share of the hyperpnea of exercise; furthermore the available data leave no provision for adjustment of pulmonary ventilation to the load placed on the muscles, but only to the rate and extent to which the limbs are moved. A recent comparison in 27 experiments upon 16 normal men of the effect upon respiration of active versus passive movements (of the same rate and extent) of one leg at the knee has shown the following: while active movements resulted in a 57 per cent increase in minute volume during the first minute, passive movements resulted in only a 34 per cent increase; with active movements respiration increased further in the second minute (to 70 per cent), while with passive movements it declined to only 20 per cent (39). In this mild type of exercise, passive movements accounted for an average of 60 per cent of the total hyperpnea. However the response was not well sustained, due either to rapid adaptation of the receptors or to the decrease in arterial pCO_2 produced by the hyperpnea; this indicates that this reflex drive must be a weak one, for strong reflex hyperpneas such as that of anoxia are well maintained despite a fall in arterial pCO_2. It is possible that if a large number of joints (ankle, knee, hip, elbow, shoulder) were brought into play at once, bilaterally, as in severe exercise, this reflex might assume more importance, especially if the arterial pCO_2 were maintained by concomitant active contractions of muscles. The experiments of Barman, Moreira and Consolazio (11, 10), purporting to show that there is little or no reflex drive during exercise, actually do just the reverse and provide experimental data minimizing the rôle played by chemical substances from the limbs. In their experiments, subjects walked on a treadmill until a steady state was reached; the average pulmonary ventilation in 11 experiments on three subjects

was 41.3 liters per minute at this time. While the subjects were still walking at the same rate, arterial and venous circulation to both legs was occluded for 1½ minutes by cuffs around the upper thighs; during this period, ventilation decreased to 38.6 liters per minute, a fall of only 2.7 liters per minute or 6.5 per cent of the steady state ventilation. Since respiration falls off by 40 to 55 per cent in an average individual in the first 1½ minutes of rest after exercise of similar severity (142, 7, 135, 101, 124) it would seem that the difference between 40 to 55 per cent and 6.5 per cent (33.5 to 49.5 per cent) could represent the contribution of reflexes from the moving limbs (or of other factors such as discomfort or pain). Further experiments should be done along this line. (Barman pointed to the intense hyperpnea occurring after release of the occluding cuffs as important evidence that the hyperpnea of muscular exercise is due predominantly to central stimulation by liberated metabolites. It must be emphasized that these conditions are far from physiological and give no information as to the situation existing normally. During complete occlusion CO_2, lactic acid and potassium may accumulate in the tissues and be swept into the re-established circulation; changes in venous pressure certainly and arterial pressure possibly also occur along with changes in blood temperature. Occlusion of the vessels during exercise cannot be counted upon with certainty to do more than establish or rule out the existence of a reflex component.)

Experiments employing electrical stimulation have shown that a marked increase in ventilation may be produced by excitation of certain sensory nerves in the limbs. Krogh and Lindhard (100), using the Bergonié apparatus for inducing muscular contraction in man by electrical stimuli, succeeded in producing hyperpnea without pain or discomfort, without a rise in alveolar pCO_2 and obviously without irradiation from the motor cortex. For corresponding quantities of oxygen absorbed, electrically induced work produced a greater hyperventilation than voluntary work; in one experiment ventilation increased to more than 70 liters per minute with an oxygen absorption of only 800 cc. per minute. This would indicate the existence of peripheral nerves or receptors capable of stimulating respiration when these are excited electrically. As a rule, however, Krogh and Lindhard found that the electrical stimulation was associated with pain or discomfort. Y. Henderson (81) found that marked hyperpnea occurred in dogs with mechanical or electrical stimulation of the central end of the cut sciatic nerve; he attributed this to stimulation of pain fibers. Faradic stimulation of both legs in human subjects increased respiration from 6.58 to 13.95 liters per minute when only moderate discomfort was experienced; when the stimulus was increased, respiration increased to 25.6 liters per minute but intense pain was perceived (20). Since pain is not ordinarily encountered in dynamic muscular exercise, these receptors would appear to be of little importance. However in static effort (34) (such as holding a weight, or supporting oneself on parallel bars) discomfort or even pain may be a prominent feature (20). For example the simple extension of one leg in mid air led to an increase in ventilation to 34.7 liters per minute at the end of the fourth minute; the subject had to discontinue the effort because of the intense discomfort (20). This may be due

to the fact that muscle spasm limits the blood flow through the muscle and thus favors the production of lactic acid and the sensation of fatigue (though the former has been doubted by Rein (131) and the latter by Jervell (93)).

3. Chemical receptors in the limbs. This raises the very interesting question as to whether there might be receptors in the limb which respond to concentrations of muscle metabolites lower than those which produce pain. It is known that chemical factors formed during muscular exercise may produce vasodilatation, pain (105), and a reflex increase in blood pressure (1); it is also known that chemoreceptors capable of stimulating respiration exist elsewhere in the body (carotid bodies (83), aortic bodies (140), lower respiratory tract (136)). The possibility of the existence of similar receptors in the limbs has been investigated in a variety of ways. Comroe and Schmidt (37) were unable to demonstrate reflex stimulation of respiration in man, cat, or dog by total ischemia or by products of muscle metabolism formed by exercise during complete ischemia; if pain was avoided exercise during ischemia produced no greater hyperpnea than exercise alone. The latter has been confirmed by Barman et al. (11); these authors, however, found a measurable increase in respiration during ischemia alone. Delucchi (48) found that the respiratory increase produced by static effort of the legs was increased when cuffs around the thighs were inflated to a pressure two-thirds that of the systolic blood pressure; since no discomfort was experienced, this could be regarded as evidence for the existence of chemoreceptors in the limbs. However the respiratory changes were very small (11–16 per cent) and at no time was the leg circulation completely occluded.

A large variety of substances including creatine, phosphocreatine, blood from exercised ischemic muscles, anoxic blood, hypercapnic blood and acidic blood, was injected or perfused intra-arterially into the limb muscles of cats and dogs without the production of any reflex stimulation of respiration (37). Moore et al. found that acids (pH 6.0 or less) and alkalies (pH 9.2 or more) injected intra-arterially in cats often produced transient reflex respiratory stimulation (116). Neutral solutions of salts produced similar hyperpnea if their concentration was great enough. Of the chlorides tested barium, rubidium and potassium stimulated in lowest concentrations; of the sodium salts tested citrate, phosphate, fluoride and lactate stimulated in lowest concentrations (the threshold for stimulation with neutral sodium lactate was 0.17 M concentration). Moore et al. have shown satisfactorily that this reflex arises in the smaller vessels of the limb and that it probably represents a response to pain. It cannot be of any physiological significance in ordinary types of exercise though it is conceivable that metabolites formed within the muscle cells might stimulate receptors there without the production of pain.

4. Reflexes from the carotid and aortic bodies. The characteristic stimulus to these chemoreceptors is a decreased oxygen tension of the arterial blood, though unusual changes in pCO_2 or pH may also lead to reflex alteration of respiration (140). While relative anoxia may exist in the exercising muscles in exhausting exertion, a drop in arterial blood pO_2 rarely occurs in healthy individuals. Himwich and Barr found that arterial oxygen content and saturation

increased in subjects performing brief periods of vigorous work (86); similar observations have been recorded by other investigators (26, 24, 87, 80). The fact that inhalation of oxygen may lead to less dyspnea and to greater capacity for exertion (82, 121) indicates that the increase in total oxygen carried by the blood (which amounts to 10 per cent during inhalation of 100 per cent oxygen) reduces the degree of local oxygen lack in the muscles; it does not indicate a reduction of carotid and aortic body activity for inhalation of 100 per cent oxygen usually leads to a slight increase in respiration in subjects with 95 to 96 per cent saturation before the inhalation (146, 159).

It is true however that arterial oxygen saturation may fall rarely following exhausting activity in normal subjects (77) and frequently following even moderate exercise in patients with cardiac disease, anemia, emphysema, tuberculosis, pulmonary fibrosis and polycythemia vera (87, 95, 78); the magnitude of the fall varies as a rule from 2 to 10 per cent, though in two patients it amounted to 21 per cent and 55 per cent. In general however, arterial anoxemia is not a factor in the hyperpnea of exercise; the increased alveolar pO_2 resulting from the increased depth of respiration appears to be sufficient to maintain or increase oxygenation even in the face of a greatly increased pulmonary circulation rate and a decreased pO_2 in the venous blood.

So far as other stimuli to these chemoreceptors are concerned the arterial pCO_2 never rises high enough under physiological conditions to reach the threshold for carotid body stimulation; consequently any effects attributed to CO_2 must be due to action upon the respiratory center (139, 140).

So far as the response of the chemoreceptors to changes in pH is concerned, it was pointed out earlier (see p. 323) that respiration may be stimulated reflexly by acidity. The only experiments reported in which pH changes were produced without concomitant pCO_2 changes were saline perfusions of the carotid bodies; in 12 dogs it was found that a decrease of 0.1 pH unit increased ventilation 10 to 50 per cent (7 observations), 0.2 unit 10 to 50 per cent (6 observations), 0.3 unit 0 to 200 per cent (6 observations) and 0.4 unit 20 to 300 per cent (15 observations (139)). Since pH changes of 0.1 have been observed frequently during exercise (16) and changes as great as 0.4 unit have been reported occasionally (103, 155), it is possible that these chemoreceptors may account for all or a large share of the hyperpnea of exercise due to increased arterial blood acidity. The above experiments were not technically perfect (phosphate buffers were used to alter the pH of Locke solutions in which both calcium and potassium chlorides were present; precipitation of calcium phosphate may have occurred thus leaving a preponderance of potassium ions, which in themselves could stimulate the carotid bodies); they should be repeated and further attempts made to determine the sensitivity of the deafferented respiratory center to pH changes (the experiments of Hooker et al. (88) were performed before the discovery of the carotid bodies).

Reflex stimulation of respiration by warming the chemoreceptors was demonstrated by Bernthal and Weeks (23) who showed a 32.6 per cent increase in respiration upon changing the temperature of the carotid body from 39° to

43°C.; this was not a panting type of respiration since both rate and depth were increased. This was confirmed by Schmidt, Comroe and Dripps who obtained an increase of 70 per cent in minute volume when the temperature of fluid perfusing the carotid bodies was raised from 38 to 41°C. The relation of this hyperpnea to the increased body temperature observed in muscular exercise will be discussed later (see p. 329).

5. Reflexes from the lungs. Since clinical dyspnea, such as that occurring in congestive heart failure, is thought to be due in large part to an increased amount of blood in the lungs (55, 46, 35), it is conceivable that this factor may be involved in the hyperpnea of severe exertion. While there is no doubt that blood is flowing more rapidly through the pulmonary vessels in view of the marked increase in cardiac output in exercise, this does not necessarily mean that there is an increased quantity of blood in the lungs. The total amount of blood in the lungs has been estimated by vital capacity determinations in man before and immediately after exercise. A decrease in vital capacity has been found occasionally, chiefly in long races of the marathon type; in a group of 20 such runners, vital capacity was 17 per cent lower at the end of the race, but the men were so fatigued that this change was due probably to physical inability to blow into the apparatus (63). The majority of investigators have found that only insignificant changes occur in vital capacity following short but strenuous exercise. Harrison (75) found changes ranging from −150 to +200 cc. in 8 individuals (average change was −6 cc.). Iglauer and Altschule found changes varying from −230 cc. to +70 cc. in 15 healthy adults; in 12 subjects the vital capacity decreased and the average change in the 15 subjects was −80 cc. (91). Similar results were reported by Levine and Wilson (104). These slight changes are within the limits of error of the method.

It is possible that a very slight decrease in vital capacity may be sufficient to set up powerful respiratory reflexes and that the method may not be sensitive enough to detect such small changes. To answer this, we can examine the relation between vital capacity and pulmonary ventilation in cardiac decompensation, for it is generally admitted that here the increased amount of blood in the lungs is a major factor in producing dyspnea. Actually we find that very gross decreases may occur in vital capacity without correspondingly great increases in the volume of respiration. Campbell found that cardiacs with dyspnea had an average vital capacity of 53.5 per cent and pulmonary ventilation of 7.2 liters per minute; normal subjects with an average vital capacity of 95 per cent had ventilation amounting to 6.9 liters per minute (32). Peabody and Sturgis averaged respiration in 11 normals (vital capacity 101 per cent) and found a volume of 7.16 liters per minute; in 11 cardiacs (vital capacity 83.6 per cent) the volume was only 9.12 liters per minute (126). Barr and Peters reported similar results (129), respiratory volume being 10.8 liters per minute in four cardiac patients with moderate dyspnea as opposed to 6.6 liters in normal subjects (12). It is probable therefore that the very small changes in vital capacity detected in muscular exercise must have little or no effect upon respiration. It must always be borne in mind however that even small reflex stimuli may produce

greater effects when the arterial pCO_2 is maintained (152, 99) as it is in muscular exercise by increased formation of CO_2; in congestive heart failure, the arterial pCO_2 is reduced (130) (128) by the reflex drive and not maintained by excess CO_2 production. On the other hand reduction in vital capacity, whether due to increased blood in lungs or to pneumothorax or to constriction of the chest by a tight swathe (153), increases ventilation entirely by an increase in rate, the depth being reduced; this rapid shallow respiration is not characteristic of the hyperpnea of exercise, in which both depth and rate are increased.

Another possibility to consider is the actual formation of edema fluid in the alveoli concomitant with a higher pulmonary blood pressure in exercise; this may be the cause of the decreased vital capacity noted at the conclusion of marathon races and of the persistent cough occasionally experienced after gruelling exercise though Gordon et al. were unable to hear rales in the chest at such times (63). It would be interesting to obtain figures for arterial oxygen saturation in these conditions.

6. Reflexes from the great veins and auricle. Bainbridge has described a cardio-accelerator reflex arising from the great veins and auricle in response to increased pressure in those structures (3). Since this reflex is an important factor in producing the acceleration of the pulse in exercise, it might conceivably operate also toward increasing respiration. Harrison (75) felt that an increase in pressure in the great veins and right auricle increased respiration by reflexes carried over the vagi but his experimental procedures are all open to question; he increased the venous pressure by rapid infusions of large amounts of fluids into the external jugular vein and by distention of the right auricle with a balloon. Respiratory volume was increased consistently by 50–100 per cent but it is obvious that too many variables existed in such experiments to permit final conclusions.

Evidence that venous pressure does rise in muscular exercise was obtained by Schneider and Collins who found that venous pressure rose as the intensity of the work increased (143). With 4000 ft. lbs. per minute the venous pressure averaged 30 per cent above normal, at 6000 it was 50 per cent and at 8000 it was 75 to 95 per cent above normal. The drop in venous pressure after the cessation of exercise followed the usual curve for the return of ventilation to normal—i.e., it dropped rapidly in the first and second minutes after exercise and then more slowly, reaching the normal level in 6 to 9 minutes (though with very severe work full recovery was delayed 22 to 27 min.). Obviously the stimulus exists in the great veins during muscular exercise if it can be shown that increased pressure within the vein (without interfering with blood flow) actually does stimulate respiration reflexly.

7. Effect of increase in body temperature upon respiration. It is known that severe muscular exercise may result in an increase in body temperature but the extent to which body temperature may rise and its effect upon pulmonary ventilation have been generally disregarded. Many investigators have reported moderate increases in body temperature during exercise (8, 21, 22, 29, 42, 108, 109, 122, 127) and Hill and Flack have reported an instance in

which the temperature rose to 40.5°C. after severe exercise. However there have been very few measurements of the effect of an increase in body temperature upon respiratory volume. The older literature contains only scattered and incomplete observations of the effect of induced temperature increase upon the respiration of resting subjects. Graham and Poulton (64) and Haldane (70) noted dyspnea when the rectal temperature exceeded 38.9°C.; at 40.2°C. the respirations were 44 per minute (64). Sutton noted an increase in respiratory rate of 5–6 per minute for every degree C. rise in rectal temperature; "the depth and energy" of respiration increased also (154). A decrease in alveolar pCO_2 during fever was noted by Haggard (68) and by Bazett and Haldane (17). However the first quantitative studies were those performed by Landis et al. (102); by the use of hot baths, body temperature was raised to varying heights. In one subject, when the rectal temperature reached 40.3°C., the ventilation was 34.4 liters per minute; at 39.7°C., the ventilation was 21.6 liters per minute, and yet the alveolar pCO_2 had fallen in these experiments to 15.6–22.6 mm. If increase in body temperature in a resting subject can increase respiration to 34.4 liters per minute in spite of a great reduction in alveolar pCO_2, it is reasonable to suppose that an even greater hyperpnea might occur if the alveolar pCO_2 were maintained nearer normal as is the case in exercise. Probably even less striking increases in temperature could contribute a large share of the total hyperpnea when the temperature rise is due to exercise.

On the basis of the available evidence, the following evaluation may be made of the contribution of the temperature rise to the total hyperpnea of exercise: The temperature rise is certainly not responsible for the onset of the hyperventilation for the onset of the former is too slow. The few continuous measurements of rectal temperature made during exercise show that temperature may rise within 1 to 2 minutes, certainly within five minutes (22, 21, 122). Since, in steady state exercise, the hyperpnea reaches its maximum before the temperature reaches its peak, the temperature rise cannot be largely responsible for the hyperpnea. Furthermore the temperature rise varies in different individuals performing the same amount of work (84). Yet it is difficult to escape the conclusion that fever must be of great importance in explaining the hyperventilation that occurs in very strenuous exertion when the body temperature rises dramatically. It is also likely that the temperature increase can explain the long recovery period following muscular exercise for the body temperature and respiration return to normal at about the same time (42), (21); except for the first three minutes after exercise (when the respiratory volume falls rapidly), the temperature and respiration curves are parallel.

Increased body temperature may increase respiration in a number of ways: *a*, via the carotid and aortic bodies (23, 139); *b*, via the cerebral centers cephalad to the medulla (see Bazett (19)); *c*, via increase in metabolism of the medullary respiratory centers (145, 18); *d*, by decreasing blood pH (149, 150), by the action of increased temperature on blood as a physicochemical system, and *e*, by peripheral reflex factors. There is least support for the last of these mechanisms though Nasset and Peters (117) found that a greater increase in respiration fol-

lowed heating both head and trunk (separated vascularly) than either alone, and Boycott and Haldane (27) reported that "warmth causes a slight fall in alveolar pCO_2 without any rise in internal (rectal) temperature."

8. Effect of adrenalin liberation. The injection of adrenalin may lead to depression (apnea) or stimulation of respiration or to both depending upon the circumstances and mode of administration. Following intravenous injection of adrenalin with an increase in blood pressure McDowall (110) observed an apnea (now known to be due largely to pressorreceptor reflexes (83)) followed by a more lasting stimulation of respiration. Griffith et al. found that adrenalin increased respiratory volume 15 to 28 per cent in chloralosed cats (65) and Schmidt (138) noted that adrenalin is an excellent respiratory stimulant in cases of depressed respiration. Starr and co-workers (151) reported that 0.67 cc. adrenalin given subcutaneously to normal human subjects increased minute volume of respiration 42 per cent (this representing a considerably greater effect than that produced by the so-called respiratory stimulants such as caffeine, theophylline and strychnine). This increase in ventilation following adrenalin may be due to the increased metabolism that it is known to produce. It may also be due to an increase in blood lactate; Courtice, Douglas and Priestley (43) noted that blood lactate rose from a normal of 10 mgm. per 100 cc. to 33–37 following adrenalin injection and similar figures have been reported by Cook and Hurst (40). In addition to these two actions, adrenalin may have a specific stimulant effect upon the central nervous system of the type seen with other sympathomimetic amines. It is unlikely that the stimulant effect observed by Starr is due to an improvement in cerebral blood flow for ephedrine raised blood pressure more but produced less respiratory stimulation; furthermore an increase in cerebral blood flow would tend to decrease respiration by reducing the amount of stimulant material within the cells of the respiratory center (61), (137).

This increase in respiration following adrenalin suggests another mechanism by which respiration might be increased even further in severe exercise. There appears to be little doubt that adrenalin liberation does occur during muscular movements in cats (33). The extent to which such liberation occurs in man has not yet been agreed upon (52); Cannon's method for determining adrenalin liberation in cats by using the rate of the denervated heart as an indicator might conceivably be applied to man in view of the recent increase in sympathetic surgery recently. There is little disagreement, though, that adrenalin does enter the circulation in exhausting exercise.

9. Effect of irradiation from the motor cortex. Krogh's hypothesis that impulses from the motor cortex radiate to the respiratory center and increase its sensitivity has not yet been tested experimentally; evidence in favor of this theory was obtained chiefly by exclusion or lack of other factors known at that time. Krogh and Lindhard (99) felt that this concept was necessary in order to explain the immediate increase in respiration that occurs within the first second after beginning exercise; that in addition it was necessary to explain the increased respiration in spite of a decreased arterial pCO_2. However the need for this hypothesis has now largely disappeared. The immediate increase in respiration

at the start of exercise may be explained on a reflex basis as demonstrated by the work of Harrison et al. (75), Comroe and Schmidt (37) and indeed by the experiments upon electrically induced work performed by Krogh and Lindhard (100); in none of these experiments was the motor cortex involved.

Krogh's hypothesis may still be needed to explain the magnitude of the full hyperpnea of exercise but further evaluation of the rôle played by the other factors concerned is needed before this can be settled. It is possible that the sum of the effects produced by afferent nerve impulses, pH changes, temperature increase, maintenance of arterial pCO_2, venous pressure rise, adrenalin liberation all acting in harmony may provide a complete explanation. At any rate further evaluation of known factors and search for new factors susceptible of experimental proof offers a more satisfying approach than acceptance of his hypothesis. The only experimental work dealing with the effects of cortical irradiation (electrical stimulation of the motor cortex of animals) upon respiration has not provided positive evidence: cortical stimulation produced either no change in respiration or an increase in rate with a decrease in depth (148). Further experiments of this sort are desirable.

10. Effect of internal acidity of cells of respiratory center. The hypothesis (first emphasized by Gesell (61)) that alterations in respiratory activity were governed not by arterial pCO_2 or pH but by the acidity of the cells of the center, was necessitated by the lack of correlation between arterial pCO_2 and pH and respiration, and the urge for a unitarian theory of respiratory regulation. Since such a theory can never be susceptible of direct proof until methods for measuring the pH of the cells of the respiratory center without injury are devised, its acceptance must depend wholly upon the lack of other satisfactory explanations. Such a lack no longer exists: the hyperpnea of anoxemia[3], fever, ether inhalation, acidosis, cardiac decompensation, and low blood pressure may now be explained in large part or wholly by new factors, usually reflex and all capable of objective proof. Likewise the hyperpnea of muscular exercise no longer requires a theoretical approach. Furthermore such a hypothesis never satisfactorily explained the hyperpnea of muscular exercise for the hyperpnea begins simultaneously with the first movements. If the center turns more acid in order to produce this initial gasp, why does it do so? It cannot spontaneously increase its own metabolism; there must be some stimulus to the center to produce this initial augmentation of activity whether it be from the motor cortex or reflexly from the limbs, and it is the origin of these stimuli that is our chief concern.

CONCLUSIONS

Among the earliest explanations proposed for the control of respiration was that of Volkmann in 1841 (158) and Vierordt in 1844 (157), who believed that respiration was regulated chiefly by means of reflexes. One hundred years later this

[3] Davenport et al. (47) observed a delayed increase in rate and minute volume of respiration occurring in unanesthetized dogs (with carotid and aortic bodies denervated) when made to breathe 9 to 18 per cent oxygen; this could be classed as an instance of increased respiration due to increased acidity centrally if all other possibilities are excluded.

explanation again seems to be a very satisfactory one, though resting now on far firmer experimental support. In the intervening years all theories dealing with the control of respiration were concerned chiefly with the dominant effect of chemical substances acting directly upon the medullary centers (133), (60), (94), (72), (61). Probably the most important discovery of the last century—more important than the finding of any new reflex or chemical stimulant—has been the realization that respiration is controlled not by reflexes alone, not by chemical stimulation of the medulla alone, but by the proper interaction of both factors. No reflex, no matter how strong, can stimulate respiration if the arterial CO_2 tension has been lowered abnormally (99, 152); no chemical stimulant, no matter how great, can produce rhythmic breathing if the medullary centers have been completely cut off from all nervous influences including that residing in the pneumotaxic center (152). Respiratory alterations in general, and the hyperpnea of exercise in particular, cannot be explained by any single simple theory but only by a consideration of a number of known and probably many unidentified factors.

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LACTATION

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The literature dealing with the various phases of lactation is far more extensive than can be adequately treated in a single review. The object in this treating therefore shall not be to consider all of the literature on the subject but rather to review the more recent contributions and refer to older literature only as it is deemed necessary to establish a foundation, or to make contrasts. It too shall be the object to make conclusions where the evidence seems to merit them and to present all recent evidence on both sides on controversial questions.

The physiological and biochemical aspects of lactation can best be treated by considering in order each of the three distinct phases it involves. The first of these is the development of the mammary glands. The second phase is the initiation and maintenance of lactation and the third is the evacuation of the milk in the gland. While endocrines are prominently involved in all three phases, biochemical aspects are the more prominent in the second phase and need consideration for a proper understanding of the lactation phenomenon.

Recent reviews by Turner (1939), Riddle (1940, 1941), Hisaw and Astwood (1942) and Petersen (1942b) deal with the relation of the endocrines to lactation. The literature pertaining to the biochemical as well as the endocrine aspects of lactation has been reviewed by Folley (1040a) and Petersen (1942a) and in book form by Espe (1941).

DEVELOPMENT OF THE MAMMARY GLANDS. The ovarian hormones are well established as the agents responsible for the mammary growth in normal animals. Although there are some exceptions, in general, the estrogens cause duct growth while progesterone brings about development of the lobule-alveolar system when administered to the ovariectomized but otherwise normal animal. Controversy exists as to the mode of mediation of mammary growth by these hormones. It is also clear that other than ovarian hormones are essential for complete mammary development. Evidence has been presented that adrenal, thyroid, and hypophyseal hormones play a part synergistically. Placental hormones are also involved. The state of nutrition must also be considered as well as the effect of male hormones.

Exceptions to the general concept. That estrogens cause only duct growth does not hold for all species or conditions. In the guinea pig Nelson (1936) and others, and in the monkey Allen (1927) and Gardner and Van Wagenen (1938) have shown that complete lobule-alveolar development was obtained with estrogen administration to ovariectomized females. In the rat small amounts of alveolar development following estrogens have been observed by Nelson (1935) and others. In the mouse Gardner (1935) and in the rabbit Lyons (1936) have made similar observations. In the ovariectomized cow, Walker and Stanley (1941) obtained apparently complete mammary development with diethylstilbestrol as did Lewis and Turner (1941a) in an ovariectomized goat.

Since diethylstilbestrol was made available a large number of studies on its effect upon mammary development has been reported. In most cases this estrogen has been administered to animals with intact ovaries, and, therefore, some of the observed effects may be due to the action of some naturally secreted ovarian hormones. It should also be pointed out that in most cases and with larger animals in particular, complete mammary development has been assumed when the lactation was copious and not by necropsy or biopsy inspection. Large mammary development in goats by administration of diethylstilbestrol has been reported by Lewis and Turner (1942b), Folley and Young (1941a) and others. Folley, Scott Watson and Bottomley (1941a, b) Reece (1943), Lewis and Turner (1942a), Walker and Stanley (1941) and others have reported upon mammary development and milk flow simulating that expected following a normal parturition to result from diethylstilbestrol administration to nulliparous heifers. Petersen and Boyd (1944) have found uniformly good response to stilbestrol administration in the nulliparous heifers but only occasionally did multiparous dry cows respond with well developed mammary glands or high lactation levels. This failure is difficult to explain as in the multiparous animal full mammary development had occurred in a previous lactation and it would seem that a subsequent lobule-alveolar development to stilbestrol should be more readily obtained. It is also noteworthy that in 5 freemartins and several nymphomaniacs with chronic ovarian cysts very little mammary development or lactation occurred.

In general estrogen administration must be more intensive and prolonged in the male than in the female to obtain the same degree of development and in some cases complete development is not obtainable. Lewis and Turner (1941a, 1942c) report some lobule-alveolar development in male mice, rats, rabbits and guinea pigs as occurring only after prolonged treatment with large doses. The same workers (1942b) were unable to obtain any significant development in the male goat even after prolonged treatment with large doses. Petersen and Boyd (1944) treated a steer and a bull for 5 months with diethylstilbestrol and obtained but slight mammary enlargement and a few milliliters of milk daily. Failure to obtain response to estrogen treatment in the freemartin, male, and castrate male could be explained by postulating an absence of progesterone in these classes. Such postulation would be difficult to reconcile with the results obtained by Walker and Stanley (1941) who obtained full development and lactation in the ovariectomized bovine. Walker (192) concluded that progesterone is not needed for complete mammary gland development in the bovine.

In the human female stilbestrol has been used successfully by MacBryde (1939) and others to develop hypotrophic breasts. Dunn (1941) has reported a case of gynecomastia in the human male as the result of oral administration of stilbestrol.

While estrogen may cause various degrees of development of the lobule-alveolar system, Selye (1940a, 1940b) found that large doses of progesterone without pretreatment with estrogens produced mammary development comparable to late pregnancy in spayed rats. Similar reports have been made by Selye, Bor-

duas and Masson (1942) and Reece and Bivins (1942) for rats and by Hartman and Speert (1941) for monkeys.

Synergism of estrogen and progesterone. The synergistic action of estrogen and progesterone upon the uterus and sexual behavior has been the subject of much study. Recently the problem of quantitative relationship of the two hormones in mammary development has been undertaken by Lyons and McGinity (1941), Scharf and Lyons (1941) with rabbits and by Mixner and Turner (1943) with mice. These reports indicate that maximal effects are obtained with 1 mgm. progesterone to 240 I.U. estrone.

Mixner and Turner (1943) postulated that estrogen acts directly upon the tissues producing hyperemia and greater vascular permeability permitting other hormones to enter the tissues in increasing amounts. The vasodilating action of estrogen in the periphery has been shown by Abramson, Zazeela and Schkloven (1941), Hechter, Lev and Soskin (1940) and others. A large number of reports on the hyperemic effect of estrogen upon the uterus is to be found. Since the mammary gland is an accessory reproductive organ the estrogenic effect upon the uterus is of special interest because similar effects upon it can be expected. Among the more recent studies Reynolds (1939, 1941) and Kerly (1940) found that with the hyperemia of the uterus caused by estrogen there was an increase in the tissue water as well as in metabolism. Increased permeability of the capillaries of the estrogen treated uterus was demonstrated by Hechter, Krohn, and Harris (1941) by intravenous injection of trypan blue.

Direct evidence for the local effect of estrogen is to be found in the several reports on its percutaneous application and observing development only in those glands so treated. Among others, such observations have been made on the mouse by Gardner (1941) and Gardner and Chamberlin (1941); on the rat by Lyon and Sako (1940); on the guinea pig by Nelson (1941a) and Jadassohn et al. (1937); on the rabbit by Lewis and Turner (1942); on the monkey by Speert (1940, 1941a) and Chamberlin et al. (1941); on the goat by Folley et al. (1940, 1941c): on woman by MacBryde (1939) and on the cow by the reviewer and Folley et al. (1941c).

It therefore appears that one of the main, if not the chief function, of estrogen is to increase the vascular system and its permeability in the mammary gland to progesterone, other hormones and metabolites. If sufficiently large concentrations of progesterone are administered enough will get through the capillaries to produce lobule-alveolar growth without preconditioning to estrogen. The inhibitory effects of excess doses of estrogen upon mammary growth reported by Gardner (1940a) are difficult to reconcile with these views.

Rôle of the hypophysis. The usual failure of obtaining mammary development by estrogen and progesterone administration in hypophysectomized animals prove without question that pituitary hormones are involved. As to the identity of these hormones there is controversy and conflicting evidence. One school contends that estrogen acts upon the hypophysis to form a duct growth factor and progesterone acts similarly to produce a lobule-alveolar growth factor. Others contend that the ovarian hormones act directly upon the mam-

mary glands but must be assisted by a number of naturally occurring hypophyseal hormones.

Since Corner (1930) first demonstrated that administration of an anterior hypophyseal extract to hypophysectomized animals permitted of mammary development numerous attempts have been made to ascertain the pituitary fractions involved. The Missouri group have been the chief proponents of the hypothesis that estrogen and progesterone act upon the hypophysis producing mammogen I and mammogen II respectively that in turn mediate the mammary growth. Evidence for support of their hypothesis is presented in a series of reports beginning with Gomez and Turner (1937a) and Gomez, Turner, and Reece (1937), that implantation of pituitaries from rats treated with estrogen caused mammary development in hypophysectomized guinea pigs while pituitary implants from nontreated rats failed. Later Gomez and Turner (1938) reported similar results on hypophysectomized rabbits and rats with pituitary implants from pregnant and non-pregnant cattle. Lewis and Turner (1938, 1939) and Lewis, Turner and Gomez (1939) reported upon the lipoid nature of the duct growth factor (mammogen I) and methods for biological assay. In the latter report they extend confirmatory evidence on the identity of the duct growth factor still maintaining the factor is lipoid in nature. In the latest report Trentin, Lewis, Bergman and Turner (1943) admit error in the original claim that mammogen I is lipoid and now claim it is protein in nature.

That progesterone acts upon the pituitary to produce a factor (mammogen II) which mediates lobule-alveolar development was proposed by Mixner (1940). Mixner and Turner (1941a) reported on a method for biological assay for the factor by the use of male mice and in 1942a on the rôle of estrogen in the stimulation of the lobule-alveolar growth. Mixner, Bergman and Turner (1942) present further claims for the specific lobule-alveolar growth factor in the hypophysis as they found no parallelism between the lactogenic, thyrotropic and gonadotropic activities and mammogenic potency. The work of Gomez (1942) lends support to the lobule-alveolar factor being a separate entity from other known hypophyseal factors. He found that a hot alcohol-ether extract of the fresh hypophysis contained the lobule-alveolar growth principle and none of the other established pituitary hormones. Mixner and Turner (1943) report more extensively on studies of the hypophyseal lobule-alveolar factor and suggest it is protein in nature.

While there is no question about pituitary hormones being essential for mammary growth there is strong opposition to the hypothesis that special hormones are formed in the hypophysis by the action of the ovarian hormones. Hisaw and Astwood (1942) conclude such a hypothesis to be untenable in view of the well established local effect of percutaneous application of estrogen to mammary glands previously cited. In further support of this conclusion may be cited the several reports failing to confirm several phases of the reports on "mammogen." Reece and Leonard (1939) found no difference in the mammary growth stimulation obtained in hypophysectomized rats in implants of pituitaries from estrogen treated and nontreated animals. Nelson (1938, 1939a)

made similar observations. The failure of Selye and Collip (1936) to obtain mammary development from pituitary implants is difficult to explain in light of the above results. Greep and Stavely (1941) failed to observe any mammogenic effect of a lipoid extract of pituitary glands according to Lewis and Turner (1938) while the lipoid extracted material exerted marked influence on mammary growth. The reviewer and co-workers (unpublished) have made similar observations. Mixner and Turner (1943) explain the failure of obtaining differences from implants of pituitaries from estrogen and nontreated animals in the hypophysectomized animals as being due to the lack of sensitivity in the operated individuals as compared to intact male mice which they used as test animals.

Successes in replacement therapy by various hypophyseal hormones strongly suggest that naturally occurring pituitary hormones act in conjunction with the ovarian hormones to produce mammary development. Although not corroborated by Nelson (1938, 1939), Greep and Stavely (1941), Reece and Leonard (1939) and others, the fact that Lewis and Turner (1939) and Mixner and Turner (1943) obtained larger amounts of duct growth and lobule-alveolar growth respectively, from the hypophysis in pregnancy than from that of non-pregnant or male animals it is possible that in pregnancy certain metabolic hormones needed for mammary development are increased in the hypophysis.

The development of the mammary gland is a growth phenomenon and would therefore be expected to involve the action of several of the principles of the hypophysis affecting metabolism. The general rôle of the hypophysis in this relation has recently been reviewed by Long (1942). Inanition, an invariable sequela to hypophysectomy, has been shown by Astwood, Geschicter and Rausch (1937) to prevent mammary development in otherwise normal rats. The work of Nathanson et al. (1939) supports, and Trentin and Turner (1941) confirmed, this finding but found that greatly increased doses of estrogen would cause mammary duct proliferation in inanition. Although inanition is undoubtedly a factor in the failure of complete mammary development in ovarian hormone administration in hypophysectomized animal it is not the only one as Samuels, Reinecke and Petersen (1941) noted no greater development in hypophysectomized rats that were force fed by stomach tube so as to gain in weight than those not so treated. In a more recent report, Samuels, Reineke and Bauman (1943) found that the weight increments in force fed hypophysectomized animals was due to fat storage. There was a loss of the nitrogen stores.

In view of these facts it is surprising that response in hypophysectomized animals should be obtained with anything less than a therapy of a complete extract of the hypophysis yet there is a growing literature reporting on varying degrees of development obtained by the administration of various hypophyseal fractions in conjunction with ovarian and other hormones. Frederickson (1939) obtained mammary development in hypophysectomized rabbits with estrogen and progesterone but was unable to obtain lactation from such glands with prolactin unless the animal was pregnant. Gardner (1940) obtained some mammary development in hypophysectomized male mice with desoxycorticosterone, progesterone, and estradiol depropionate. Nelson (1941b) reported

that the andrenotropic hypophyseal fraction caused slight to marked mammary development in castrated hypophysectomized rats of both sexes. Chamorro (1940) obtained lobule-alveolar development by administration of desoxycorticosterone acetate and estrogen to hypophysectomized male rats. Gardner and White (1941) obtained mammary development from estrogen and lactogen concluding that estrogen sensitizes the gland to the action of the lactogen. Later Gardner and White (1942) found that when an adrenotropic extract was administered together with estrogen and lactogen still better development was obtained. A saline extract of pituitaries, ineffective by itself, greatly enhanced the action of the estrogen and lactogen. This further emphasizes the probability of estrogen working in conjunction with hypophyseal hormones. That the growth hormone is involved is indicated by a number of reports. Using hypophysectomized animals, Reece and Leonard (1941) found that growth hormone augmented the mammary growths to estrogen. Later (1942) these workers obtained good lobule-alveolar development in hypophysectomized rats by the administration of growth hormone and testosterone propionate. Leonard (1943) found estradiol dipropionate alone, if injections were begun one day after hypophysectomy, would stimulate end buds but was ineffective 7 days after the operation. Samuels, Petersen, Reinecke and Bauman (unpublished data) too have observed marked beneficial effects from the growth hormone but contend other anterior pituitary hormones are also essential for complete development.

Further evidence that the lactogenic hormone plays an important rôle in development in the normal animal is indicated by a number of recent reports. Astwood (1941) reported that lactogen or a hypophyseal hormone closely associated with it, and for which the term luteotrophin is suggested, maintains and regulates the corpus luteum function. That luteotrophin is distinct from the hypophyseal luteinizing hormone seems to be well established by the reports of Astwood (1941), Evans, Simpson, Lyons and Turpeinen (1941) and Evans, Simpson and Lyons (1941). From these works it appears that the luteinizing hormone only promotes growth of the corpus luteum and the luteotrophic hormone causes it to secrete progesterone. Lyons, Simpson and Evans (1941, 1942, 1943) have added confirmatory evidence and by use of highly purified lactogenic hormone have identified it as the luteotrophin, as has Tobin (1942).

Not only are the results of Lyons (1941), using the guinea pig in certain stages as a test animal for the lactogenic hormone, explained by these new discoveries but they add much to the understanding of the mammary development in pregnancy. The corpus luteum becoming persistent (in some species there is exception) is stimulated by the lactogenic hormone to secrete progesterone which in turn acts upon the mammary gland, conditioned by estrogen, to stimulate lobule-alveolar growth. Other hormones from the hypophysis and other endocrine glands also play more or less important rôles.

Rôle of the placenta. That the placenta is capable of taking over the mammogenic functions of the hypophysis is evidenced by at least three recent reports. Desclin (1939) hypophysectomized pregnant guinea pigs and found no signs of

atrophy when autopsied 9 to 12 days later while in males where the glands had been developed by estrogen complete atrophy was observed following hypophysectomy. Newton and co-workers (1939, 1941) likewise observed normal glands in hypophysectomized pregnant mice. In the first report they expressed the fetuses leaving the placentas *in situ* and found no evidence of atrophy whereas when the placentas as well as the young were expressed atrophy of the mammary glands was complete. This experiment should rule out the hypophyseal secretion of the fetuses as the causative factor. In the latter report some of the hypophysectomized animals parturated normally and the glands were filled with milk but all young died because of lactation failure.

Rôle of the thyroid gland. The reports on the rôle of the thyroid gland in mammary development are conflicting. Dragstedt et al. (1924) reported complete mammary development in pregnant dogs deprived of their thyroids and parathyroids when tetany was prevented. Leonard and Reece (1941) reported thyroidectomy to enhance mammary growth in both normal and castrated female rats with or without estrogen treatment. Smithcors and Leonard (1942) observed thyroidectomy of male mice to inhibit mammary growth. A lesser effect was noted in castrated males. Gardner (1942) reported orally administered desiccated hyroid to stimulate mammary growth in male mice but not in castrated males. Mixner and Turner (1942b) noted improved mammary development in thyroidectomized mice when thyroxine was administered with estrogen and progesterone but not with estrogen alone. In cattle Spielman, Petersen and Fitch (1944) observed much less mammary development in thyroidectomized cows and heifers than would be expected in normals. Petersen, Knodt and Ludwick (1944) have observed that no apparent mammary development is obtainable by stilbestrol administration in thyroidectomized myxedemic heifers but with simultaneous thyroid administration normal response was obtained.

These conflicting results cannot be adequately interpreted on the basis of available experimental evidence. The complete development observed by Dragstedt et al. (1924) in thyroidectomized pregnant dogs might be accounted for by the thyroxine secretion of the developing fetuses. Spielman, Petersen and Fitch (1944) observed no mammary development in pregnant myedemic heifers until about midterm. The beginning of mammary development roughly corresponded to the disappearance of myxedema both of which were attributed to the secretion of thyroxine by the developing fetus. Because of the rôle the thyroid plays in general metabolism and growth it would be reasonable to believe that it plays a part in optimal mammary development.

The rôle of the adrenals. The function of the adrenals in normal mammary development is speculative. That the adrenal hormones can effect mammary development is unquestionable, but whether or not they do so in the normal intact pregnant animals is not definitely proven. The beneficial effects of hypophyseal adrenotropic fractions upon mammary development in hypophysectomy observed by Nelson (1941), Gardner and White (1942) and others indicate that probably normal function of the adrenals is essential for complete develop-

ment. That desoxycorticosterone is the mammogenic factor of the adrenals has been shown by its effects when injected. Van Heuverswyn et al. (1938), Speert (1940b) and others obtained full mammary development in guinea pigs and monkey from desoxycorticosterone injections as did Chamorro (1940) with hypophysectomized male rats when also estrogen treated. Leonard and Reece (1942), however, failed to obtain mammary growth with desoxycorticosterone in hypophysectomized rats with or without estrogen. That desoxycorticosterone has the mammogenic properties of progesterone is established. Mixner and Turner (1942b) found it to be about one-half as potent as progesterone with mice as the test animals and may therefore augment progesterone under natural conditions. Interesting from a speculative point of view is the report by Beall and Richstein (1938) of finding progesterone and allopregnanolone in the adrenal. Of speculative interest is also the report of Butcher (1939) that adrenalectomy enhanced the mammary gland development in underfed albino rats.

Effect of other steroids. A comparatively large number of steroids have lobule-alveolar growth stimulating properties. A number of studies on the mammogenic effect of testosterone propionate has been reported recently. Folley, Guthkelch and Zuckermann (1939) and Van Wagenen and Folley (1939) obtained good lobule-alveolar growth in monkeys. Reece and Mixner (1939) obtained complete lobule-alveolar development in spayed rats and initiation of secretion. They also report a 40 per cent increase of the lactogen content of the hypophysis as a result of testosterone propionate injections. Lacquer (1942, 1943) observed lobule-alveolar growth in rats and sufficient lactation for foster sucklings to obtain milk. Noble (1939) obtained duct and lobule-alveolar growth in intact duct and lobule-alveolar growth in intact rats by testosterone propionate injections but only nipple growth resulted when the animals were ovariectomized and hypophysectomized. Leonard and Reese (1942) likewise failed to obtain mammary growth with testosterone propionate in hypophysectomized rats. Forbes (1942) studied the age differences in response to testosterone propionate implantation finding no mammary response in prepubertal, a moderate response in pubertal and complete response in post pubertal male and female rats. This age difference in response may account for some of the conflicts in the literature as to observed effects of various treatments.

Reese (1941) found that androsterone was ineffective while Mixner and Turner (1942c) found testosterone and testosterone propionate ineffective and dehydroandrosterone effective in producing mammary development in spayed mice.

Mixner and Turner (1941b) reported pregneninolone to have lobule-alveolar stimulating effects. Selye (1941) reported acetoxy-pregneninolone to be only slightly less effective than desoxycorticosterone. Mixner and Turner (1942c) however, found it to be only about one-fifth as active. Using spayed mice as assay animals these workers have reported the mammogenic properties in terms of progesterone for the following steroids to be: desoxycorticosterone 1/2; dehydroandrosterone 1/3; acetoxypregnenolone 1/4; methyl testosterone

1/25; and testosterone and testosterone propionate as being ineffective. Unless there is a species difference their failure to get mammary growth response from testosterone and testosterone propionate is difficult to explain as all other work indicates that these substances are effective in promoting lobule-alveolar growth.

LACTATION. With the exception of the last two years when relatively few publications have appeared a large literature has developed on the complex phenomenon of lactation. In general the contributions in this field can be rather sharply divided between those that deal with the endocrine and those that deal with the biochemical and other physiological aspects of the problem. While the work in recent years has added much for a better understanding of this complex phenomenon much is still obscure and considerable controversy exists on several details. Some of the controversy comes from the rather sharp cleavage between those working on the endocrine and those working on the other physiological aspects of the problem. Because of this fact and that there is more or less interdependence of all the physiological aspects of the problem it is deemed advisable to forsake the idea of limiting this review to the endocrine phases and include the biochemical and other physiological problems as well. Such a plan obviously necessitates the forsaking of some of the details that a more limited approach would permit.

For ease in treatment the discussion will be divided into the following four heads: A, endocrine factors; B, time when milk is secreted; C, milk and blood equilibrium factors; and D, synthesis of milk.

A. Endocrine Factors. It is well known that the mammary gland may be well developed, such as in pseudopregnancy or by the administration of mammogenic principles without lactation occurring. This fact is taken as *a priori* that other factors than those necessary for mammary development are essential for lactation. In search for these factors studies have led to the possible rôles played by the hypophysis, thyroid, adrenals and pancreas.

1. *The anterior pituitary.* Stricker and Grueter (1928) started the long list of investigations of the hypophyseal function in lactation when they reported initiation of lactation in pseudopregnant ovariectomized rabbits by the injection of an aqueous extract of the anterior hypophysis. Since then efforts have been directed toward the identification of the hypophyseal fractions that are lactogenic. While major efforts have been expended in studies on the so-called lactogenic factor (prolactin) conclusive evidence is at hand to indicate other hypophyseal fractions contribute in a major way. The reports on effects of various fractions are difficult to evaluate in many instances because of the impurity of the substances used. Until very recently none of the so-called AP fractions have been free from contamination and now only one, prolactin, is obtainable, in pure form.

Although much of the effect upon lactation attributed to prolactin is due to substances with which the preparation used was contaminated a number of facts point to its being one of the hypophyseal lactation factors. While Gardner and Turner (1933) and Nelson (1934) have proposed other methods for analysis the standard test animal is the pigeon and the test is based upon the crop growth

stimulation. It is significant that the prolactin content of the hypophysis varies as to species, physiologic stage and age, first reported by Bates et al. (1935), and since added to by many. Reece and Turner (1937a) found the hypophysis of dairy cattle to contain significantly more of the hormone than those of beef cattle and less in calves than in older animals. In another report (1937b) these workers found the administration of estrogen to increase the prolactin content of the hypophysis. In another report, Reece and Turner (1937c) found the stimulus of nursing to increase the hormone. Reece (1939) working with guinea pigs found the greatest hypophyseal concentration of the hormone in lactation with successively decreasing amounts for late pregnancy, estrus, early pregnancy and diestrus. Holst and Turner (1939) found no increase in early pregnancy, little increase in late pregnancy, and large increases following parturition in rabbits and guinea pigs. In pseudopregnant rabbits, Meites and Turner (1942a) found no increase in the prolactin to that of the normal. Lewis and Turner (1941b) were able markedly to increase the prolactin content of rat pituitaries by the administration of stilbestrol and (1942b) obtained similar results with estrone injections in male rabbits. Prolactin has also been identified in the urine of lactating women by Lyons and Page (1941), Turner and Meites (1941) and Ehrhardt and Voller (1939); in the blood and urine of pregnant and lactating mares by Leblond (1937); in placentas by Turner and Meites (1941) and in the urine of babies secreting witch's milk by Lyons (1937a).

The differences observed by Chance et al. (1939) in the prolactin content of the hypophysis in different species shows no relation to their lactation capacities. They report but little of the hormone in the hypophysis of the horse, most in the sheep, with man, swine and cattle in between in ascending order.

Since lactation is abolished by hypophysectomy it is only natural that replacement therapy in the hypophysectomized animal should be used in the earlier work in a study of the lactogenic hormone or hormones of the hypophysis. Among the successes in maintaining lactation in the hypophysectomized animals by administration of pituitary extracts are the reports of: Riddle et al. (1933) for rats, Lyons et al. (1933) and Housay (1935a, 1936) for the dog; Mephail (1935) for the cat; Stricker and Grueter (1928) for the rabbit. Nelson and Gaunt (1936) and Gomez and Turner (1936) found that a more purified preparation of prolactin failed to initiate lactation in hypophysectomized guinea pigs and pointed the way for search of other hypophyseal factors involved in lactation.

The effect of anterior pituitary extracts administered to larger normal animals has been reported by a number of workers. Grueter (1930), Evans (1933) and Asdell et al. (1936) observed marked stimulation of lactation in goats as did Kabak and Kisilstein (1933) in sheep. In the cow Grueter and Stricker (1929), Asimov and Krouze (1937), Folley and Young (1937, 1938, 1939) and others have reported marked increases in milk production subsequent to the injection of a crude extract of the anterior hypophysis.

Stockklausner and Daum (1932) are the only ones to have reported a decline in lactation in cows following hypophyseal extract administration. In all

experiments of this kind it has been found that lactation increases are the greatest when treatment is made in the declining phase of the lactation. It should also be noted that the benefits from treatment are as a rule only temporary even when treatment is continued over a period of time (Folley and Young, 1940, 1941a).

As with hypophysectomized laboratory animals when the more purified prolactin was administered, Folley and Young (1939, 1940, 1941b) and Sykes et al. (1942) found the benefits to lactation are greatly diminished. The reports of Bergman and Turner (1940) and Lyons (1937b) in which they obtained engorgement of the glands of pseudopregnant rabbits with prolactin treatment may seem confusing. However, these had intact hypophyses presumably capable of producing the other necessary hormones. There is also a question as to the quantity of milk that can be produced under those conditions and certainly the preparations used were not pure prolactin as now produced by the method of Li et al. (1940 to 1941).

From these reports it is obvious that the anterior pituitary contains lactogenic principles and it is also equally clear that prolactin is not the only lactogenic hormone. A question may even be raised as to the function of prolactin in lactation as it is not peculiar to the lactating female. It is found in pituitaries from all animals and classes. Rabald and Voss (1939) have shown it to be present in the liver and Lyons (1937) found it in the urine of men. Prolactin in lactation is therefore not a problem of quality but rather one of quantity.

The lactogenic hormone has been used extensively in attempts to increase lactation in the puerperium with mostly negative results. Kayser (1940), Stewart and Pratt (1939), Werner (1939) and many others were unable to observe any beneficial effects from the administration of relatively large doses and frequently undesirable reactions were noted. Kenny and King (1939) and Kurzrok et al. (1939) are among the few reporting benefits to lactation in women from lactogen.

The effect of prolactin upon carbohydrate metabolism is suggested by Folley and Young (1941b) as possibly being more important to lactation than the direct action upon the mammary gland. In a recent report Schooley et al. (1941) reemphasized the rôle of prolactin in enlarging the abdominal viscera, promoting body growth and increasing appetitite in the pigeon. The luteotrophic properties of prolactin recently discovered and reviewed in the previous section is also a matter to consider in the re-evaluation of the rôle of prolactin in lactation. This is especially so in light of the report by Lyons (1941) where lactation was induced in normal female guinea pigs by injection of prolactin commencing not later than the third day following estrus. When the injections were started on the fourth day following estrus, or later, lactation was not initiated. These results are difficult to explain as the assumption must be that the action of the prolactin must be through the corpus luteum and there is no evidence that this body is necessary for lactation. Ovariectomized animals often have been brought into and maintained in lactation.

The adrenotropic hormone has recently been shown to be essential and without it lactation can neither be initiated nor maintained. Some of the successes with

so-called prolactin preparations must be laid to the contamination of such preparations with the adrenotropic fraction.

2. *The adrenals.* It has been known for some time that ablation of the adrenals causes complete inhibition of lactation (Carr, 1931; Swingle and Pfiffner, 1932; Gaunt, 1933, and many others). Administration of cortical extracts prevented lactation failures and before the purification of the various adrenal cortical hormones led to speculation as to the active principle. A number of reports including Nelson and Gaunt (1936, 1937a, 1937b) Schultze (1937) and Gomez and Turner (1936, 1937b) have obtained complete relief from adrenalectomy symptoms by administration of extracts of the adrenal cortex. This has led to speculation as to the active principle. Brownell et al. (1933) proposed a special lactation hormone in the adrenals and more recently Spoor, Hartman and Brownell (1941) have reported that an extract from the adrenals, not prolactin, is more powerful in pigeon crop stimulation than the adrenal (cortin) hormone. Gaunt and Tobin (1936) and Hurst, Meites and Turner (1942), however, were unable to detect any lactogenic hormones in the cortical extract.

With the advent of the isolation and purification of the different cortical principles new light has been shed on the fractions involved in lactation. Gaunt (1941a, 1941b) has shown that desoxycorticosterone is incapable of maintaining lactation after adrenalectomy. As a matter of fact, there was evidence that it had an inhibitory effect. Nelson and Gaunt (1937a) however, observed that a little lactation could be induced in hypophysectomized guinea pigs with lactogen and a high salt diet. Again these workers (1937b) found in the rat that desoxycorticosterone had a slightly beneficial effect upon lactation. They concluded as did Levenstein (1937) that alterations in the electrolyte and water balance such as are affected by desoxycorticosterone have an adverse effect upon lactation.

That carbohydrate metabolism disturbance is more important than the water and electrolyte balance while suggested by Gomez and Turner (1936, 1937b) was demonstrated by Gaunt, Eversole and Kendall (1942) and Nelson, Gaunt and Schweizer (1943). They showed that lactation could not be induced in the hypophysectomized guinea pig with lactogen and desoxycorticosterone but with the carbohydrate metabolism fraction, 17-hydroxy-11-dehydrocorticosterone and lactogen copious lactation was induced. They also report that desoxycorticosterone had an inhibitory effect upon lactation in the guinea pig. The report of Lazt (1941) that a crude AP extract plus prolactin caused lactation in adrenalectomized rats is difficult to reconcile.

The influence of the hypophysis upon the adrenal is well known. Apparently there is no reciprocal effect, at least insofar as prolactin is concerned. Meites, Trentin and Turner (1942) have reported that the lactogen content of the rat hypophysis is not affected in adrenalectomy. Jones and Nelson (1942) propose a significant relation between the hypophysis and the adrenals. They found that stilbestrol acts upon the hypophysis to stimulate secretion of the adrenotropic hormone and it in turn causes increased secretion of the adrenal carbohydrate metabolism hormone.

As in other cases in which endocrines are involved the pregnant state exerts an influence. Tobin (1940) found that adrenalectomy before or during pregnancy does not necessarily cause abortion but lactation failed after parturition even when the glands were well developed.

Since progesterone can substitute for desoxycorticosterone to some extent maintenance of the corpus luteum during pregnancy may be a factor in the well being of the pregnant adrenalectomized animal. The effect of secretions by the placenta and fetus, however, should not be overlooked.

On the basis of the work reported to date a conclusion is warranted that the adrenals serve in lactation primarily by the secretion of the carbohydrate metabolism hormone 17-hydroxy-11-dehydrocorticosterone. Also that desoxycorticosterone is necessary to prevent water and electrolyte disturbances but in larger quantities has an inhibitory effect upon lactation.

3. *The thyroid.* The relation of the thyroid to lactation continues to be the subject of investigation by means of thyroidectomy, hypophysectomy, and thyroid administration to normal lactating animals. Considerable disagreement exists as to the rôle of this gland in lactation. Dragstedt et al. (1924) found that thyroidectomy did not influence lactation in the dog provided tetany was prevented. Nelson and Tobin (1937), Karnofsky (1942) and Nelson (1939b) concluded similarly for the rat. Folley (1938, 1942), Prenheim (1940) and Folley, Scott Watson and Amoroso (1942), however, report significant diminution in milk secretion following thyroidectomy in this species. In the goat Trautmann (1919) reported marked decreases in thyroidectomized animals while Hibbs et al. (1941) obtained lactation for more than a year following thyroidectomy. In the thyroidectomized cow, Graham (1934a) reported only a slight depression in milk yield while Spielman et al. (1944) noted sharp reduction in milk production.

In thyroidectomized animals no evidence exists that the administration of either thyroxine or the thyrotropic hormone assists in the initiation of lactation.

In the administration of thyroid and thyroxine to intact lactating animals the large number of reports warrants the conclusion that such administration will increase milk yields and fat percentage. Graham (1934a, 1934b); Folley and White (1936); Herman et al. (1938); Hurst et al. (1940); Dastur and Smith (1939); Ralston et al. (1940); and Reinecke and Turner (1942) have reported increases in both milk yield and fat percentage following thyroid or thyroxine administration to cows. Jack and Bechdel (1935) observed only increased milk yield. De Fremery (1936) observed a decrease in milk yield in goats as a result of thyroxine injections probably due to too large doses. Probably for the same reason Grumbrecht and Von Düsterlo (1937) obtained decreased lactation in the guinea pig following injection of the thyrotropic hormone. While Bergman and Turner (1943) could not demonstrate any increase in the thyrotropic hormone in the hypophysis of pregnant and lactating rabbits, in contrast Folley and Young (1939, 1940) found hypohyseal extracts containing the thyrotropic principle more effective in increasing milk yields than preparations without this factor.

It is apparent that the thyroid is not essential for the initiation of lactation and therefore is not lactogenic in the strict sense. That the thyroid hormone exerts a significant influence upon lactation must also be concluded. Its influence on lactation is undoubtedly due to its effects upon metabolism. Since milk fat yields are affected more than total milk the report of Dastur and Smith (1939) that thyroxine administration causes a decrease in phospholipids, glycerides and fatty acids in the blood are of interest. In these animals the milk fat was increased by 40 to 50 per cent.

Dinitrophenol, while having marked effects upon the metabolic rate was found by Graf et al. (1940) to have no effect on milk yields in cows when administered in small doses. With large doses there was a marked diminution of milk yields as well as marked changes in milk composition. Brower and Martin (1938) made similar observations on goats.

While as early as 1924 Dragstedt et al. reported that it was necessary to control tetany resulting from ablation of the parathyroids in order to maintain the level of milk yield in the dog it remained for Folley (1941a) and Folley, Scott Watson and Amoroso (1942) to demonstrate that the parathyroids are essential for optimum milk yields.

4. *Rôle of pancreas.* No recent reports on the relation of the pancreas has been found. The work of Nelson, Chaikoff and Lyons (1933) indicates that some lactation may take place in the depancreatized dog although it is greatly impaired. At this time there is no evidence that insulin has any direct action upon the mammary gland but that its effect upon carbohydrate metabolism is responsible for the influence on lactation.

5. *Starting lactation.* In normal conditions the mammary gland develops during pregnancy, and lactation as it is usually understood begins following parturition. Much evidence *vida supra* has been presented that the lactogenic hormone is greatly increased at the time lactation begins. As to the mechanism responsible for the release of the lactogenic hormones at this time little concrete evidence and much speculation has been advanced. These speculations fall into 4 main groups as brought out by Meites and Turner (1942c) as follows: 1, corpus luteum; 2, the placenta; 3, mechanical distention of the uterus and 4, the estrogen.

One of the oldest postulates is that the corpus luteum prevents onset of lactation. Expression of the corpus luteum in goats by Drummond-Robinson and Asdell (1926) and in rats by Selye et al. (1934) resulted in lactation with the obvious conclusion that this body was the inhibitory factor. Anselmino and Hoffman (1936) and Folley and Kon (1937) and others have failed to note any inhibition of lactation from the injection of progesterone into lactating animals indicating that if the corpus luteum is the responsible body the inhibition must be due to some other secretion than the progestational hormone. The hypothesis, however, is untenable in the light of the well known fact that copious lactation may prevail during pregnancy with well developed corpora lutea in woman, the cow, and the goat.

That the placenta inhibits lactation is supported by the work of Frankl

(1923), Nelson (1934) and Smith and Smith (1933) who observed inhibition of lactation when placentas were retained or transplanted. In contrast, however, Litt (1933) with rabbits and Selye et al. (1934) with mice were unable to observe any inhibitory effect upon implantation of placentas. Further evidence against this hypothesis is the fact that placentas which are frequently retained in cows do not inhibit lactation and also the well known fact that in several species copious lactation may occur during the entire pregnancy.

Mechanical distention of the uterus as the inhibitory factor is subject to the same objections as a satisfactory explanation of the problem. Selye (1934) and Selye et al. (1934) found filling the uterus with parafin following removal of the young by Caesarian section prevented lactation. Bradbury (1941) and Greene (1941) using the same technique could not confirm this observation. They explained the apparent failure to be due to the effects of the operation as, following recovery, from the effects of the operation they observed lactation in animals with their uteri filled with paraffin.

That estrogen will inhibit lactation in animals is well established by a large number of reports but much larger doses are needed for this effect than would be present in pregnancy. Among the many reports dealing with the experimental inhibition of lactation are those of De Jongh (1933) and Robson (1935) for the mouse; Reece and Turner (1937), Bacsich and Folley (1939) and Folley and Kon (1937) for the rat; and Folley (1936, 1941c) for the cow.

Among the large number who have reported success in inhibiting lactation and preventing painful post-partum engorgement of the breasts are: Kurzrok, Birnberg and Livingston (1942), Jeppson, Kasabch and Kanter (1942), Diddle, Nagyfy and Sells (1942), Diddle and Keettel (1942) and Barnes (1942). The reports of Arabanel and Goodfriend (1941) and Arabanel and Klein (1941) confirm the effects of estrogen on preventing painful postpartum engorgement of the breasts but they question the inhibitory effect upon lactation claiming that lactation will continue if the infants nurse. Novak (1943) on the basis of clinical experience concurs with this view.

Those who have observed inhibitory effects of estrogen upon lactation found that treatment to be effective must begin in the early puerperium. When lactation had become established no inhibitory effects were noted. Administration of diethylstilbestrol to lactating cows by Folley et al. (1941d) and Spielman et al. (1941) caused no diminution in milk yields but enriched the milk in fat, protein and sugar. The androgens, particularly testosterone, have also been used clinically to inhibit lactation and to relieve painful post-partum engorgement of the breasts with varied success. Jeppson et al. (1942) reported testosterone propionate was helpful in inhibiting lactation but of no value in relieving painful engorged breasts in the puerperium. Fleischner and Kushner (1941), however, reported that testosterone offered complete relief of pain and engorgement in 68 per cent of their patients and Hellman and Auer (1941) found it to inhibit lactation in 35 out of 77 women.

Nelson, Gaunt and Schweizer (1943), Gaunt, Eversole and Nelson (1942) and others have observed that desoxycorticosterone has an inhibitory effect upon

lactation and that guinea pigs are more sensitive than are rats. Since estrogen, testosterone and desoxycorticosterone all stimulate mammary growth and all have been reported to inhibit lactation, Nelson, Gaunt and Schweizer (1943) advance the idea that all substances that stimulate mammary growth have an inhibitory effect upon lactation. The fact that large doses of estrogen failed to inhibit lactation in milking cows, that there is a question about estrogen and testosterone inhibiting lactation in women and that progesterone which stimulates lobule-alveolar lactation growth raises a question about the validity of such a statement under all conditions.

The fact that the inhibitory effect of estrogen upon lactation in women has been reported to be far more effective in the early puerperium than when lactation becomes established points to a greater sensitivity of the mammary gland before initiation of lactation to the inhibitory substances. However, prepartum inhibition of lactation is not the only factor involved. It is well known that cows may be brought into full lactation before parturition by the stimulus of milking. To the reviewer it seems more logical to look for some stimulating factors as being responsible for postpartum lactation than to attempt explaining the phenomenon by the removal of prepartum inhibitory factors.

Turner and Meites (1942c) proposed the two primary requisitions for copious lactation as being: (a) well developed mammary gland and (b) high lactogen content of the anterior hypophysis. Both of these are satisfied before parturition. Lyons (1937b) and Bergman and Turner (1940) have shown that milk secretion begins some time before parturition as the enlargement of the mammary gland in late pregnancy is due to the engorgement of the alveoli by secretory products. The characteristics of colostrum milk add further support to a prepartum secretion because its chemical and physical properties are those expected as the result of equilibria forces exerted upon retained milk (Petersen and Rigor, 1932a). In view of these facts it is apparent that postpartum milk flow is not due to an initiation of secretion at that time but rather a beginning of ejection of the alveolar contents. Ely and Petersen (1941) advanced the hypothesis that ejection of milk from the alveoli was due to oxytocin secreted reflexly by the posterior pituitary as a result of the milking stimulus. Since oxytocin is secreted during parturition this will account for the initiation of milk flow which is kept up by the subsequent stimuli of nursing or milking. Reece and Turner (1936, 1937c) observed that the act of nursing maintained high levels of lactogen in the pituitary, and Hooker and Williams (1940) reported that lactogen injections retarded the rate of mammary involution. Stewart and Pratt (1941) for women and Williams (1941) for mice have added additional evidence for the stimulating effects of nursing upon lactation. Other facts supporting the hypothesis that postpartum lactation is due to the introduction of stimulating factors rather than the abolition of inhibitory factors will be dealt with in the section on "let down of milk."

The question may be raised as to how nursing stimulates the production of lactogen in the pituitary suggested by Meites and Turner (1942c). While they suggested a direct effect, that is the nursing stimulus, reflexly stimulated the

secretion, two other alternatives exist. Since nursing stimulates the liberation of a posterior pituitary hormone it in turn may stimulate secretion of the lactogenic hormone. Another alternative is that evacuation of the mammary gland may be the stimulating factor.

B. Time of Milk Secretion. Since no recent reports have appeared on the subject it must be taken for granted that the problem of the time when milk is secreted during the interim between milkings is settled. The older view advanced by Isaachsen (1923), Maxwell and Rothera (1915) and more recently by Ingelbrecht (1935) that a large portion of the milk is secreted during the nursing act is untenable. The view advanced by Petersen, Palmer and Eckles (1929a), Swett, Miller and Graves (1932), Gowen and Tobbey (1928), that the milk is secreted in the interim between milkings, now prevails. The study of pressure relations to the rate of milk secretion by Petersen and Rigor (1932b) and Garrison and Turner (1936) indicates that the rate of milk secretion decreases as the milk accumulates in the alveoli. The work of Shaw and Petersen (1940) shows that there is no uptake of blood precursors for milk during the milking and indicates a complete cessation of milk secretion at this time. The phenomenon of let down of milk in many cases has been mistaken for milk secretion.

C. Equilibrium between Blood and Milk. It is well known that milk and blood have the same total osmotic pressure 6.6 atmospheres. What is not generally appreciated is that the two are not in equilibrium and it may well be that the lactogenic hormones have something to do with the establishment of the forces necessary to maintain a state of non-equilibrium between them. As yet Simms (1931) is the only one to have studied the problem of differences between the constituents of blood and milk as related to equilibria phenomena. According to him, on a molar basis the ratios of concentration in milk as compared to blood are: fat, 20; sugar, 40; potassium, 7; calcium, 14; magnesium, 4; and PO_4, 7 to 1. Blood on the other hand contains 2, 4 and 8 times as much protein, chlorine and sodium, respectively, as does milk. It is significant that none of the major constituents in milk has the same concentration as a similar constituent in the blood.

For other constituents the concentrations in the blood and milk are identical. This has been shown by Peskett (1934) to be the case with urea and probably the same with uric acid, creatine and creatinine. Kolda (1926) reviewed an extensive literature on the passage of blood constituents of a dietary origin into the milk and Petersen and Brereton (1942) have reported on several inhaled substances passing into the milk. The mammary gland therefore acts as a permeable membrane to many blood substances while for others there is selectivity. One can only speculate on the nature of the selective mechanism. The fact that sodium bicarbonate is not found in milk can be accounted for by postulating that the mammary membranes are impermeable to it. Postulation of partial permeability will account for those substances found in lesser concentration in milk than in blood. Wright (1928) has presented evidence that calcium levels may be built up in the milk by combining with casein and phosphorus and thus maintain a Donnan equilibrium insofar as this constituent is concerned. Other

constituents not in equilibrium cannot be explained on this basis and what is more must await further work before a satisfactory explanation can be advanced.

The best evidence for the existence of forces that prevent milk from coming into equilibrium with blood is that several conditions will set into motion mutual exchanges between blood and milk constituents. Conditions that will bring this about are: infusion of various substances into the lactating gland, development of intra-alveolar pressures of sufficient magnitude and by general physiological disturbance including gland pathology.

Petersen and Rigor (1932c) and Garrison and Turner (1936) have studied in detail the effects of irrigating the cow's mammary gland with distilled water and various concentrations of solutes. Filling the gland immediately after milking with distilled water reduced subsequent milk flow but slightly and had little effect upon the character of the milk. With increasing salt or sugar concentrations in the infused fluid there were progressive decreases in the amounts of secretion and alterations in character. In general the values for protein, total solids, mineral matter, catalase and pH increased and lactose decreased. The reviewer and co-workers have accumulated considerable unpublished data on the effects of blood serum, gums, gelatin, starch, fats, oils, various other agents thought to have therapeutic value in the treatment of mastitis and have found that only water or water with sulfanilamide have little or no disturbing effect upon either the quantity or quality of milk. Hucker and Lee (1932) reported a low sodium chloride (0.12 per cent) solution as well tolerated. While it is strange that an isotonic salt solution should have such a depressing effect upon lactation when infused, stranger still is the marked deleterious effect of infusing back into the gland the freshly drawn milk as reported by Jackson and Rothera (1914), Davidson (1926) and Garrison and Turner (1936). The disturbance by reintroduction of the milk was of a similar magnitude to that of physiological saline solution.

The early attempts by Neusch (1910), Isaachsen (1923) and Tgetgel (1926) in determining the secretion pressure developed in the udders of cows by means of manometers connected with the gland sinus were in error. They either measured the hydrostatic pressure of the milk in the sinus or the much greater pressure from the let down of the milk. Petersen and Rigor (1932b) and Garrison and Turner (1936) attempted to measure the maximum intra-alveolar pressure against which milk would be secreted by insufflating the udder with air by the former, and oxygen by the latter. The former found 25 mm.Hg and the latter 40 mm.Hg as the maximum pressures against which milk will be secreted. It is also established that as the intra-alveolar pressures increased the rate of milk secretion decreased. The increased milk production observed by dairymen by more frequent milking is explained by such a procedure keeping the intra-alveolar pressure to a minimum and getting the optimum rate of milk secretion. Ludwick, Spielman and Petersen (1941) milked one side of the udder three times daily to gain 16 per cent in milk production over the side milked but twice daily.

The effect of leaving the milk in the mammary gland for varying periods of time after the maximum intra-alveolar pressures had developed was found by these workers to alter the composition of the milk. Apparently as soon

as milk secretion ceases because of pressure the milk begins to come into equilibrium with the blood. Colostrum like milk can be produced at any time of the lactation by suspending milking for a long enough period of time. Undoubtedly natural colostrum is the result of filling the alveoli with secretion products some time before parturition so that partial equilibrium with the blood has been established. Petersen and Rigor (1932a) showed that once the milk had been changed by refraining from milking, several days of regular milking were required before the milk became restored to normal. Porcher and Muffet (1930) observed that the casein decreased and the globulin increased in milk retained in the gland beyond the normal length of time. If milking is delayed sufficiently long all of the milk in the gland is resorbed and involution occurs (Wayne, Eckles and Petersen, 1933).

Graf, Ludwick and Petersen (1940) and Brower and Martin (1938) administered dinitrophenol to cows and goats, respectively, and found a marked disturbance in the gland resulting in it becoming permeable to sodium bicarbonate and the milk became alkaline. Other changes in the direction of colostrum milk were also observed. Apparently this drug had a direct effect upon the permeability of the gland cells. Changes in the composition of milk in chronic mastitis are also in the same direction to indicate that any disturbance to the secretory cells affect their permeability to various milk and blood substances.

. *D. The Synthesis of Milk.* If the number of contributions to the literature is a criterion the study of the synthesis of milk has suffered more than its share of loss due to the war because very few reports on the subject have appeared during the past two years. Immediately preceding this period, by the same criterion, there was great activity in this field. One is impressed with the efficiency of the mammary gland. Graham, Houchin, Peterson and Turner (1938) calculated that only 10 per cent of the total energy uptake as determined by analysis of incoming and outgoing blood in the goat was used by the gland itself. Gaines (1928) calculated the overall efficiency of lactation to be 52.6 per cent in the cow or that 52.6 per cent of the energy intake above maintenance requirements is returnable in the milk.

Several people have studied the ratio of blood flow through the gland to the amount of milk secreted. Determining the mammary gland uptake of glucose in goats by arterio-venous difference and assuming quantitative conversion of glucose into lactose, Graham, Jones and Kay (1936) calculated 500, and Lintzel (1934) 256 volumes of blood per volume of milk. Using the thermostromuhr, Graham (1937b) found a ration of about 250 to 1 which is in agreement with the values obtained earlier by Jung (1933) using a stromuhr. Shaw and Petersen (1938c) calculated a ratio in the cow of 387 to 1 on the basis of calcium uptake and 391 to 1 on the combined glucose and lactic acid uptake assuming the latter two are quantitatively used for the formation of lactose.

The lack of agreement among the reports cannot be fully explained. Part may be due to a state of excitement in the animal when samples are taken or measurements made. Shaw and Petersen (1939) observed as much as 14 per cent change in blood concentration due to excitement when blood samples are

taken. Reinecke, Stonecipher and Turner (1941) recognizing the effects of excitement have proposed anesthetizing the animal when observations are made but in so doing probably introduce other errors such as changed circulation and metabolism. Since there is a question about the disposition of glucose in the mammary gland calculations based upon its quantitative conversion into lactose are subject to question.

Fat metabolism in the mammary gland. Milk fat is quantitatively the most variable constituent in milk. Because of its great variation in milk from the same individual it is suggested that its synthesis in the mammary gland is governed by some factors not involved in the secretion of other milk constituents. The problem of fat synthesis in the mammary gland has been attacked by many workers and from a number of different angles. The first attempt at determining the blood precursors of milk fat was made by Meigs et al. (1919) using the Kaufmann and Magne (1906) technique in which they concluded that the blood phospholipids were used for the formation of milk fat. Using the arterio-venous difference technique, Blackwood (1934) Lintzel (1934) Graham, Jones and Kay (1936), Maynard, McCay, Ellis and Hodson (1938) Shaw and Petersen (1940) and Voris et al. (1940) demonstrated that the mammary gland takes up neutral fat from the blood. These workers as well as Aten and Hevesy (1938) were unable to detect any uptake of phospholipids.

Hilditch and Paul (1936) and Hilditch and Jones (1936) suggested that the short chained fatty acids result from a breakdown of the long chained acids taken up from the blood. The work of Shaw and Petersen (1940) demonstrating that more than enough neutral fat is taken up by the mammary gland to account for the milk fat supports this view. Additional support to the theory that the short chained acids of milk fat result from partial oxidation and reduction from longer fatty acids is found in the reports of Gowen and Tobbey (1928) and Petersen, Palmer and Eckles (1932b) who found the fat of a lactating mammary gland to be intermediate in composition between milk fat and body fat while that of the non-lactating gland was similar to body fat. The identification of lipase by Kelly (1938, 1943) and of free fatty acids in the basal part of the secretory-epithelium by Kelly and Petersen (1939) indicated that neutral fats are hydrolyzed in the gland.

Because they observed the respiratory quotient of the lactating gland to be greater than unity, Graham, Peterson and Houchin (1938) and Reinecke et al. (1941) concluded that fat must be formed from carbohydrate. Shaw (1939) also observed the R.Q. of the mammary gland is greater than unity. The reviewer and co-workers have extensive unpublished data obtained from both the intact animals and from excised glands perfused according to the technique of Petersen, Shaw and Visscher (1941) that also show a respiratory quotient above unity. While the classical interpretation is that R. Q's above unity indicate fat formation from carbohydrate this is not necessarily so, as pointed out by Soskin (1941).

The recent studies showing that the lactating mammary gland uses β-hydroxybutyric acid adds speculation as to its function in milk fat synthesis. Shaw and

Knodt (1941a) observed that β-hydroxybutyric acid is constantly used by the mammary gland in normal conditions and Shaw (1942) reported more than twice as great usage in a cow suffering from ketosis. He calculated that all of the oxygen uptake by the gland would be required for the oxidation of the β-hydroxybutyric acid taken up by the gland in ketosis and about 37 per cent in normal animals. In another report Shaw and Knodt (1941b) calculated that the β-hydroxybutyric acid taken up by the lactating mammary gland is sufficient to account for all short chained fatty acids in the milk up to C_{14}. More recently Shaw and Petersen (1943) studied the utilization of ketone bodies using the perfusion technique. It was found that the β-hydroxybutyric acid utilization increases with increased amounts in the blood and aceto-acetic acid is not used.

On the basis of the evidence at hand it is certain that blood phospholipids are not the precursors of milk fat. In spite of the contrary indications in the observed R.Q. it is probable that there is no conversion of carbohydrate to fat in the mammary gland because more than enough neutral fat is taken up to account for the milk fat. The part that β-hydroxybutyric acid plays in the fat metabolism of the mammary gland is only speculative at this time.

Carbohydrate metabolism in the mammary gland. Among the many that have shown that the mammary gland takes up glucose from the blood are Blackwood and Stirling (1932), Lintzel (1934), Graham et al. (1936, 1936, 1938) and Shaw, Boyd and Petersen (1938). The assumption is that the blood sugar is used for lactose formation. That lactic acid may also be a blood precursor for lactose is indicated by the reports of Graham (1937) and Shaw, Boyd and Petersen (1938) who showed that lactic acid is also removed from the blood. These workers calculated that the lactic acid plus glucose removed from the blood would be about enough to account for the lactose in the milk. Powell and Shaw (1942) later questioned that lactic acid is removed from the blood under normal conditions as they could not detect any uptake unless cows were excited. Reinecke, Williams and Turner (1941) reported that appreciable glycoproteins were removed from the blood and suggest the sugar is split off to become available for the carbohydrate metabolism in the mammary gland.

To what extent lactic acid and the sugar part of glycoprotein are involved in lactose synthesis is not known but that glucose is the chief precursor of lactose is obvious. Lowering of the blood sugar by insulin administration is reported by Petersen, Hewitt, Boyd and Brown (1931), Brown, Petersen and Gortner (1936b), Gowen and Tobbey (1931b) and others to cause a decrease in the lactose content of the milk. Hypoglycemia produced by the administration of phloridzin has been reported by Patton and Cathcart (1911) and Gowen and Tobbey (1931) to produce similar results. Similarly reduction in the lactose content of milk was observed by Gowen and Tobbey (1931a) and Overman and Wright (1927) in the hypoglycemia produced in inanition. Hyperglycemia is reported to increase the lactose content of the milk when produced by intravenous injections of glucose by Nitzescu (1925) but not by Brown, Petersen and Gortner (1936c) or Petersen and Boyd (1937). Whitnah, Riddell and Hodgson (1933) increased the milk sugar by the introduction of large amounts of sugar into the

stomach of cows by stomach tube. Both hyperglycemia and increased lactose in the milk were obtained by Jones (1935) through thyroxine administration and by Bottomley, Folley, Walker and Scott Watson (1939) by subcutaneous implantation of epinephrin tablets. Intramammary duct injections of glucose by Brown, Petersen and Gortner (1936c) produced hyperglycemia but no increase in lactose content of the milk in the glands not injected. Although the blood sugar was not determined the administration of stilbestrol to lactating cows was found to increase the lactose content of milk by Spielman, Ludwick and Petersen (1941) and Folley, Scott Watson and Bottomley (1941d).

While it is obvious the blood glucose is the chief precursor of milk sugar the mode of its conversion is little understood. Grant (1935) obtained lactose synthesis by incubating mammary tissue slices with glucose and with glucose and galactose (1936). Petersen and Shaw (1937) could not demonstrate lactose synthesis from glucose and mammary gland tissue unless lactic acid was added. Since the mammary gland contains 0.2 per cent glycogen (Petersen and Shaw, 1938) it would seem that it must play a part in lactose synthesis. Whatever the intermediate steps are can only be conjectured. That the phosphorylated breakdown products of glycogen occurring in muscles are not involved is indicated by Grant (1936) who could demonstrate no lactose formation by tissue slices when hexose monophosphate or a phosphoglycerate was added.

That the systemic carbohydrate metabolism is altered during lactation is shown by Cahane (1938) when following removal of the mammary glands of guinea pigs he noted increases in blood glucose and liver glycogen and a decrease in muscle glycogen. His postulation that the lactogen hormone causes a conversion of glycogen to glucose is significant in light of one of the modes of action of lactation hormones.

Nitrogen Metabolism in the Mammary Gland. Very little as yet is known about the nitrogen metabolism in the mammary gland. The conclusion by Cary (1920) supported by Blackwood (1932) that blood amino acids are the precursors of milk proteins has proven to be at least partly wrong. Graham, Houchin and Turner (1938), Graham, Peterson, Houchin and Turner (1938), Shaw and Petersen (1938a, 1938b) and Reinecke, Peterson, Houchin and Turner (1939) agree that there is uptake of blood amino acids by the mammary gland but the quantity is entirely too small to account for the milk proteins. Graham, Houchin, and Turner (1937) and Shaw and Petersen (1938a) did not simplify the problem by discovering that nearly sufficient urea is eliminated by the mammary gland to account for the nitrogen contained in the amino acids taken up. Of course the source of the urea is not known. Since Shaw and Petersen (1938b) described arginase in active mammary gland tissue one would expect some of the urea to be formed by the action of this enzyme on arginine.

That blood globulin passes through the mammary cells in small amounts is unquestionable because of the identity of blood and milk globulin. That large quantities are taken up is claimed by Graham, Peterson, Houchin and Turner (1938) and Reinecke, Peterson, Houchin and Turner (1939). Jackson and Gortner (1938) also suggest that blood globulin is a significant precursor of milk

proteins. They found globulin to predominate in lactating while albumin predominated in nonlactating bovine mammary glands. Reinecke, Williamson and Turner (1941) reported that appreciable glycoprotein material is taken up. The evidence points strongly to blood globulin being a prominent precursor of milk proteins. How it is converted into casein or lactalbumin or what part the amino acids play in the synthesis is not known. It is significant that according to Shaw and Petersen (1938b) uric acid, creatine and creatinine are neither taken up nor eliminated.

THE EJECTION OR "LET DOWN" OF MILK. The phenomenon of "let down" of milk normally occurring as the result of the nursing or milking act has been subject to much speculation. By many it was believed to be the result of a rapid secretion of milk brought about by the nursing stimulus. (See section, "Time of Milk Secretion.") Others interpreted the phenomenon as being due to the forcing out of the milk from the alveoli and finer ducts. Zwart (1916) was one of the first to draw attention to the distinction between milk secretion and the let down of milk but offered no explanation as to the mechanism involved in the latter.

Failure properly to understand the phenomenon has led to a great deal of confusion. As the nervous system is involved many have sought "secretion" nerves which Ribbert (1898) proved was not necessary for milk secretion as a transplanted rabbit gland secreted milk. Yet many have sectioned the nerves to mammary glands in small animals and have noted adverse effects upon lactation (Ingelbrecht, 1935; Selye, 1934; Selye and Collip, 1936; Bacq, 1932; Selye, Collip and Thomson, 1932; Cannon and Bright, 1931; and Simeone and Ross 1938). Ely and Petersen (1941), however, noted no difference in the milk yield of the two halves of the cow's udder in one of which the nerve trunk had been cut and Labate sectioned all known nervous pathways to the ovary and uterus without any effect upon lactation. Cannon and Bright noted the adverse effect to come in the next lactation following the operation. Ingelbrecht resected the spinal cord in rats so that the front breasts remained innervated and made the significant observation that the young put on the posterior glands died from starvation when shielded from the sight of the mother. When young were placed on the front glands simultaneously with those on the rear, both groups obtained milk. Ingelbrecht concluded that most of the milk was secreted as a result of the nervous stimulation in nursing.

That the nervous system is involved in lactation is not to be questioned but recent work has proven that its function is chiefly if not entirely concerned with the expression of the milk from the alveoli and finer ducts and not in its secretion. Among the many theories advanced to account for the phenomenon is that of Zeitzmann (1922) who postulated a contraction mechanism in the gland as being responsible for retaining the milk. Hammond (1936) postulated that the nursing stimulus reflexly caused engorgement of the gland with blood to squeeze the milk out of the alveoli. The turgid condition of the gland at the beginning of nursing, held in support of this theory, is not due to engorgement with blood but rather to the distention of the lower portions of the gland with milk forced

out of the alveoli. Ely and Petersen advanced the hypothesis that the "let down" of milk in cows was due to a reflex complex involving the sensory nerves in the skin of the teats and gland which when stimulated caused the secretion of the oxytocic principle of the posterior hypophysis, and this hormone in turn caused contraction of the musculature in the mammary gland described by Swanson and Turner (1941). Many have shown the posterior hypophyseal hormones to have milk expressing properties, beginning with Ott and Scott (1910) and later Turner and Slaughter (1930) and many others, but Ely and Petersen (1939, 1941) were the first to advance the idea that the "let down" of milk was caused by the oxytocic principle. They noted that about 45 seconds intervened between the application of the stimulus and the let down of milk which agrees with the time required for the principle to be carried by the blood to the mammary gland in the cow. Petersen and Ludwick (1942) demonstrated that the principle causing "let down" of milk was humoral in nature by showing that blood taken from cows stimulated to let down their milk caused "let down" of milk when perfused through excised glands while blood taken from cows not so stimulated failed. In experiments using the perfusion technique, Petersen (1942c) added further evidence that the oxytocic principle is the causative agent. While the pitressor principle caused "let down", also shown by Turner and Cooper (1941), it required larger quantities and had marked vasoconstrictor effects not noted in the blood from cows stimulated to let down their milk. The observations of Miller and Petersen (1941) that incomplete evacuation of the gland resulted if milking was delayed after the stimulus for "let down" had been applied, also supports the claim that oxytocin is the active factor for it is well known that this factor is rapidly destroyed in the blood.

The importance of considering this phase of lactation is obvious for if the milk is not removed from the gland the effects will be the same as when there is a failure of milk secretion. That some of the so-called failures of lactation in experimental animals may be due to failure in the "let down" of milk is indicated by the report of Gomez (1939, 1940) who obtained milk secretion in post gravid hypophysectomized rats by the administration of extract of the posterior pituitary. The reported successful lactation in rats by Smith (1932) and in dogs by Houssay (1935a) when the posterior hypophysis was removed is difficult to explain.

As to be expected, Ely and Petersen (1941) found states of excitement may completely inhibit response to the milking stimulus. It is not improbable that lactation failures, particularly in neurotic women, may be due to a lack of response to the stimulus of nursing. Knodt and Petersen (1944) have studied three cases of precipitous drops in the lactation in cows which were due to incomplete evacuation of the gland at milking as when the oxytocic hormone was injected, lactation levels were returned and maintained. They also were able to level off the lactation curves of cows in the declining phase of complete evacuation of the gland at each milking by the use of oxytocin. This suggests that the decline in milk yields and even the ultimate involution of the mammary gland may be due to a diminishing response to the milking stimulus and less complete evacuation of the gland. That the mammary gland of the goat involutes in

about 30 days following suspension of milking was reported by Turner and Reinecke (1936). They also observed that involution was retarded in the side where milking was suspended when the other side was milked. One explanation for this retardation may well be that with each milking there was a partial evacuation of the milk in the alveoli thus permitting limited secretory activity. Additional support to the proposition that incomplete response to the milking stimulus is the cause of declines in the lactation curve is found in the report of Miller and Petersen (1941) that milk yields declined rapidly when evacuation of the gland was incomplete as a result of delay in milking following the stimulation for the "let down."

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MAINTENANCE OF NITROGEN BALANCE BY THE INTRAVENOUS ADMINISTRATION OF PLASMA PROTEINS AND PROTEIN HYDROLYSATES

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Studies of nitrogen balance have been used extensively in determining the effectiveness with which various forms of protein nourishment meet the protein needs of the body. Without going into a critical analysis of the accuracy of such a yardstick, it may be assumed that, apart from growth, the value of any protein administered for nutritional purposes must depend to a large extent upon whether it is able to maintain nitrogen balance. As a corollary to this metabolic point of view it may be further assumed that maintenance of nitrogen balance depends upon the presence of all of the essential amino-acids. The studies reviewed herein deal almost entirely with the administration of protein nourishment in the form of plasma protein or hydrolyzed protein through the intravenous route. The importance of this method has become apparent only in the past few years and will probably increase as the necessity of administering protein nourishment parenterally becomes more widely recognized. In order to evaluate correctly their influence on the level of nitrogen output, only studies will be included in which the injected material formed the *sole source of nitrogen*. As will be noted, this criterion has been maintained as far as protein hydrolysates are concerned; mostly for historical interest a few of the early studies on blood and plasma transfusions in humans and in animals were included in which protein food was also given by mouth. No attempt will be made to review a considerable literature on nitrogen balance following the parenteral, usually subcutaneous, injection of various serums and other proteins usually for purposes of immune or non-specific protein therapy; much of this data is old and has been reviewed elsewhere (29). Of historical interest (8) are a few early reports describing artificial nutrition, by which was meant the subcutaneous injection of milk and other substances; the term intravenous nutrition was apparently used even earlier (1899) in a report (27) which also contains much of this earlier literature.

In the following discussion various observations will be divided arbitrarily into those in which plasma and those in which hydrolyzed protein was injected; each group is further subdivided into observations made upon animals and upon humans.

PLASMA PROTEIN. Studies on the maintenance of nitrogen balance following the introduction of plasma protein intravenously include the use of whole blood as well as plasma transfusions. Fractions of the plasma proteins have been injected in the human but thus far no data on their metabolic behavior are available. In the case of whole blood the hemoglobin of the red cells represents over twice as much protein as that in the plasma part of the blood, yet it probably does not enter into the behavior of protein metabolism at least within the period of days usually involved in nitrogen balance studies inasmuch as hemoglobin is not catabolized as long as the red blood cell remains intact, and with compatible

blood, destruction of red cells is a slow process. A few observations are included in which a solution of hemoglobin has been injected. However, it is obvious that the injections of plasma alone are of greatest interest from the point of view of protein nutrition and most of the reports do deal therewith.

Animal experiments. Largely of historical interest are two studies reported in 1875. In the first (19) two fasted dogs were each given a large transfusion of defibrinated dog blood and the urine collected and studied for several days before and after, but only the urea output was measured. A 10 to 20 per cent increase in urea was observed continuing for several days. However, a control injection of 300 cc. of glucose intravenously showed a similar increase, suggesting an untoward reaction, possibly from infected material. These dogs were then given horse serum intravenously, but toxic symptoms occurred in two and there was an even larger increase in the urea output. Egg white was given to one dog and it was followed by albuminuria. In the second report (43) two dogs were given defibrinated blood, one by mouth, the other intravenously. The urinary nitrogen was increased in each experiment, but much greater in the former than in the latter.

The earliest modern study on nitrogen balance as influenced by the intravenous injection of plasma in animals was not made until 1908 (29). Both swine and dog serum were used; with the former, single doses in 7 dogs produced severe reactions and a pronounced negative balance due to a tremendous increase in nitrogen output in the urine, accounting for most or all of that administered. Dog serum was then given in 3 single doses to 3 dogs and the nitrogen output followed for 5 days. In 2 of the dogs which were fasted the nitrogen output did not change during the day of injection, even though the amount of nitrogen injected was only slightly greater than the total daily nitrogen output during control periods so that positive balance was achieved. Moreover, there was no increase in output during the post-injection period. In the third dog which was fed and already in positive balance, there was likewise no change in the urinary nitrogen during or after the injection, thus increasing the positive balance on the day of injection by the amount injected. More variable findings are reported in another series of experiments (21) on 4 fasting dogs given single direct whole blood transfusions amounting to 100 to 200 cc. In the first experiment the nitrogen output increased gradually so that it doubled on the 25th day after transfusion and then increased again for a day following a second transfusion. In the second dog the nitrogen output remained unchanged for 2 days. In the third animal it remained unchanged for 6 days and then fell. In the fourth animal it increased after 5 days and then fell below the pre-injection level. In still another dog, which was fed, nitrogen output increased gradually by 40 per cent on the 11th day. No explanation was offered for the considerable variations, although no mention was made in regard to possible reactions. Much more consistent findings were reported in another study (2) on 4 dogs kept on a nitrogen free diet and given serum intravenously on 3 successive days, the amount of nitrogen equalling the nitrogen output in the 3 previous days. In 2 experiments with dog serum there was no change in the nitrogen output during the 3

days, thereby producing a positive balance; moreover there was no increase in nitrogen output during the 2 days following the injection. In the other 2 experiments with horse serum there was an increased nitrogen output of about 20 per cent during the 3 days of injection in one case, but only a slight increase in the other instance during 2 days; positive balance was not observed in either case.

Two other papers should be classified with these early studies, although they were performed in 1936 and 1938. In the first (28) six dogs were put on a non-protein diet and given small amounts (60 to 80 cc.) of citrated dog blood per day for from 1 to 5 days, and studied for 2 more weeks. There was no change in the nitrogen output during the injection or post-injection periods except in one animal weighing 9 kgm. given a larger amount of blood (130 and 155 cc. on 2 successive days). However, even here the increase was very slight and occurred on the second day; the amount of nitrogen in the plasma was, however, insufficient to expect positive nitrogen balance. In the second paper (39) similar experiments were carried out on 3 dogs kept on a low nitrogen diet previous to the injection of whole citrated blood. In only one of these experiments was there a significant increase in the nitrogen output during the days in which a transfusion was given. Changes after the transfusions were finished were hard to estimate in view of the fact that the animal was put on a full diet at the conclusion of the transfusion period.

The probable influence of incompatible blood on nitrogen balance formed the subject of a study in 1932 (24) in which whole dog blood was given to dogs kept on a protein-free diet. Experiments were divided into 2 groups depending upon whether the blood was absolutely compatible or whether it showed evidence of incompatibility. The experiments were carefully carried out for several days before and after a single transfusion of 100 cc. of whole blood. With this amount it was impossible to expect positive nitrogen balance, calculating as nitrogen intake only the plasma protein. However, the difference in the nitrogen output in the 2 groups was significant. Seven experiments with blood which did not match resulted in a 10 to 20 per cent increase of the urinary nitrogen output developing within a few days, but which returned to normal in 6 to 8 days. By contrast there was not only no change in the nitrogen output in the urine, but indeed a slight fall in 5 experiments, with transfusions of blood which matched completely. In each group both citrated as well as direct transfusions were employed with similar results.

In the past decade there has been an increasing number of studies on nitrogen balance in dogs given plasma or serum intravenously. In 1934 experiments were described (23) in which heparinized dog plasma was injected into 4 dogs on a nitrogen-free diet. In the first dog negative balance occurred during the first of two 7 day injection periods, even though the nitrogen injected exceeded the output during the preliminary 5 days; however, in the second 7 day period a positive balance of nearly one gram was observed because the nitrogen output fell in spite of an increased intake; in the post-injection period of 5 days there was only a slight increase in the nitrogen output. In the other 3 dogs consistent positive

balances were achieved in 2 successive 7 day periods with no increase in the post-injection period of 5 days. By contrast a similar experiment in which the plasma was given by mouth rather than vein was followed by negative balance due to an increased nitrogen output, though the excretion in the final control period dropped to the same level as that of the preliminary control period. In a paper the following year (37) further similar experiments were reported from the same laboratory. In the first of 2 experiments nitrogen balance was barely missed during two 7 day periods and one 4 day period, due undoubtedly to the small dose of plasma given; in none of them did the nitrogen output reach the preliminary level. In the second experiment nitrogen balance was achieved during one 8 day injection period; in the 5 day period following the nitrogen output fell. By contrast, however, one experiment is described in which intravenous horse plasma for one 7 day and one 3 day period led to a progressive increase in the nitrogen output lasting into the post-injection period; moreover the dog became sick and died and at autopsy showed a definite nephritis. In another experiment dog plasma was injected intraperitoneally on each of 8 days without any increase in the nitrogen output, producing a positive balance of over one gram a day; however in the 5 days following injection there was an increase of over 25 per cent in the nitrogen output. Horse serum given intraperitoneally in another experiment resulted in a slight increase in the urinary output of nitrogen during the 7 day injection period, a fall in the first and an increase in the second of two 4 day post-injection periods. Nitrogen balance was not achieved; however, the dose of nitrogen was much less than the output during the preliminary period. Hemoglobin was given intravenously in one experiment in amounts of less than half of the nitrogen excreted during the preliminary period. Nevertheless, a 25 per cent increase in the nitrogen output occurred during two 7 day periods which fell in the 5 day post-injection period to a much lower level. Hemoglobin given intraperitoneally in one experiment in larger amounts almost equal to the nitrogen output in the preliminary period resulted in a 50 to 75 per cent increase in the nitrogen output during 2 periods of 7 and 4 days respectively. However, in the post-injection period of 4 days the output fell almost to the preliminary level.

The probable influence on nitrogen balance of untoward reactions following plasma transfusions is revealed in subsequent similar observations from the same laboratory (10). Three intact dogs were studied while on a non-protein regime. In the first animal positive nitrogen balance was maintained in 7 out of 9 two day periods during which the amount of plasma injected each day was but slightly larger than the level of output in the preliminary period. In the entire 18 days the total positive balance was +3.2 grams. Moreover, in the 3 two day periods following cessation of the transfusions there was a definite fall in the output of nitrogen below the preliminary level. However, in the second dog during 4 two day periods plasma injections produced an increasing output of nitrogen in the urine resulting in an astonishing negative balance of over 39 grams, or almost 5 grams a day even though the total nitrogen injected was a little over half of the amount excreted during the preliminary period. That the plasma produced intoxication was shown by rapid loss of weight during this period and the excre-

tion of tremendous amounts of creatin and uric acid in the urine. During 7 out of 10 subsequent 2 day periods, this negative balance changed to a positive balance presumably because the dietary intake was altered from plain sugar by mouth to a fuller but still non-protein diet. Nevertheless, the positive balance during this second period was not very great, 2.4 grams for the entire 20 days, although the intake was increased during this period. In the third dog plasma injections also produced evidence of intoxication as shown by frequent vomiting even though there was no increase in the output of creatin and uric acid. A negative balance during each of 7 two day periods totalled 12.4 grams for the 14 days, or nearly a gram per day. The amount of plasma injected each day was somewhat larger than the nitrogen excreted during the preliminary period. The intake of food in this experiment consisted entirely of sugar by mouth; however, during 3 two day periods after discontinuance of the injections, the administration of plasma and sugar by mouth was followed by a fall in the nitrogen output to the preliminary control period.

In another paper (32) from the same laboratory two dogs on a low protein diet were each given plasma for 2 days without provoking any increase in the nitrogen output in the urine even though the amount injected was greater than the preliminary nitrogen output, thus achieving a pronounced positive balance. In still another report (31) plasma was injected during two 7 day periods in dogs being depleted by plasmapheresis on a protein-free diet. A positive balance of 6.3 and 3.3 grams was observed during these periods even though the amount of nitrogen injected was not much greater than the previous output. However, the urinary nitrogen increased 3 and 4 times during the subsequent periods due partly, if not entirely, to a corresponding increase in the oral intake of protein. In still another report (38) dog plasma was given to an Eck fistula dog on a low protein diet, kept anemic by repeated bleedings. During two 7 day periods 20 grams of nitrogen were retained, or almost 1.5 grams a day on an intake of less than 3 grams a day as plasma. Even if the output of nitrogen in the hemoglobin and plasma of the blood is subtracted, a good positive balance was achieved.

Other workers have described somewhat similar experiments in which much larger amounts of plasma were injected (15) in dogs rendered hypoproteinemic by three weeks of a non-protein diet. During the fourth week daily injections of citrated dog plasma were given totalling about 5 grams of nitrogen per day or about 3 or more times the previous level of nitrogen output in the urine. During this week of injection the nitrogen output remained unchanged over the preliminary control period so that a remarkably positive nitrogen balance of nearly 3 grams a day was achieved. However, during the subsequent 2 weeks during which there were no injections the nitrogen output increased remarkably, wiping away much of the nitrogen retained during the injection period. By contrast, similar experiments in which hydrolyzed protein was injected as described later (17) were not followed by such an increased urinary output of nitrogen.

Studies in the human. The earliest study of nitrogen balance following intravenous administration of blood in humans was apparently reported in 1921 (7). Four patients with pernicious anemia were observed during many 3 day

periods. In one period daily transfusions of 350 to 500 cc. of defibrinated whole blood were given, alternating with periods in which no blood was injected. The patients were on controlled full diets and in positive nitrogen balance. Findings were variable but there was sufficient increase in the nitrogen output during the transfusion periods to produce slight negative balance if one calculated as nitrogen intake only the plasma of the transfusion and not the hemoglobin in the red cells. On analyzing the data, however, it seemed significant that the largest nitrogen output in the urine occurred during days in which the transfusion had produced a febrile reaction, whereas in one patient showing practically no febrile reaction, there was no change in nitrogen output during the transfusion periods, thus increasing the degree of positive balance. In contrast to these findings is another study a few years later (45) in which 6 patients with pernicious anemia were given a single transfusion of 500 to 750 cc. of blood and the urinary nitrogen observed for one day before and many days afterwards. The food nitrogen intake was kept constant throughout by a regulated standard diet; however, in the one day preliminary measurement only 2 of the 6 patients were in positive balance before the transfusion, the other 4 being in slightly negative balance. The transfusions in all cases produced a negative balance even if one took into account the nitrogen of the injected plasma, due to an increased output of nitrogen which reached its highest point on the second or third day, but returned to normal on the fifth day. The magnitude of this increase varied between 2.4 grams to 4.6 grams of nitrogen per day over the control output of slightly over 6 grams. There was also a coincident increase in the uric acid excretion. No mention is made as to the manner in which the blood was given, whether it was citrated, or as to the presence of reactions. That no hemolytic reactions occurred is indicated by the fact that a well sustained increase in red cell count and hemoglobin was recorded in each case. The reported values for plasma proteins were normal or slightly high previous to the transfusions and showed no change afterwards. At about the same time another study appeared in which 6 infants and children were given transfusions of whole blood, washed red cells or plasma, all citrated (36). It is difficult to evaluate the data in this study particularly if an attempt is made to discount the effects of untoward reactions which apparently did occur; for example, following one whole blood transfusion hemoglobinuria was observed. Moreover, no data on nitrogen balance can be obtained, inasmuch as only the urea of the urine was determined. Nevertheless, it is of some interest to note that the output of urea in the urine following the injection of plasma was definitely increased in at least 2 cases. In one of these, a 17 months old infant, there was a rise from 0.80 gram per day on 4 successive days after the injection of 55 cc. of plasma containing 0.590 gram of nitrogen, returning to normal after an excess of 0.642 gram of urea had been excreted or more than the total amount of nitrogen that had been injected. However, in another instance in a one year old infant there was no change in the urea output after the plasma transfusion. In 2 instances red cell suspensions were injected without any change, whereas in 3 instances the injection of whole blood was followed by a slight increase. Following the injection of 60 cc. of saline as a control there was

no change in the output of urea for 3 days, though the authors stated that the total nitrogen did increase.

The next report appeared quite recently (25) and describes 2 cases which were studied before, during and after daily injections of bovine and human plasma intravenously. In the case of the bovine plasma the daily injection of 6.15 grams of nitrogen for 5 days resulted in an output of 6.91 grams or a negative nitrogen balance of 0.76 gram. The caloric intake as glucose during the entire experiment was 1500 per day and no food was taken by mouth. Although there was a slight negative balance the output of nitrogen did not increase and indeed was below the 7.88 gram level of the preliminary 5 days, and was only slightly above the post-injection 6.45 gram level of 5 days. Thus a positive balance may have been achieved with slightly larger injections. On the other hand, the patient in whom human plasma was injected went into a pronounced daily positive balance of +1.23 grams during the 5 days in which plasma containing 5.6 grams of nitrogen was injected. The output during the preliminary period was 6.62 grams and the post-injection period of 4 days was 3.39 grams. In other words, the plasma transfusions were accompanied and followed by an actual fall in the urinary excretion of nitrogen.

INJECTIONS OF HYDROLYZED PROTEIN. In the following observations the proteins used were digested by different methods and include a few studies with mixtures of pure amino-acids. A recent review (33) on intravenous alimentation with amino-acids contains a discussion of the different types of protein hydrolysates. Observations on the metabolic effects of injections of single amino-acids will not be reviewed; discussion will be confined to studies made with hydrolyzed protein or mixtures of amino-acids given intravenously for nutritional purposes and will be divided into animal and human observations.

Animal experiments. As early as 1889 an undetailed report (35) appeared in which a digest of casein and other proteins, prepared by an apparently mild hydrolysis with alkali was injected intravenously without producing albuminuria or toxic symptoms, which had been noted following the injection of whole casein solutions. However, nitrogen balance was apparently not achieved inasmuch as the administration was followed by an increased output of non-protein nitrogen in the urine. In the paper on nitrogen balance following plasma injections, already discussed (29), are described 2 experiments in which an alkali hydrolysate of milk protein was injected into dogs with some evidence that much of the injected nitrogen was retained though not enough was given to expect positive balance. This was in 1908. Four years later (6) an enzymatic hydrolysis of meat was used which, however, evoked violent reactions including diarrhea and albuminuria when injected intravenously in dogs and a pronounced negative nitrogen balance. The following year a successful experiment was reported (22) in a 15.5 kgm. goat which was maintained in positive nitrogen balance for 16 days; all fluid and food were given by a continuous intravenous drip containing glucose, salts and an enzymatic hydrolysate of meat in which 10 to 15 per cent of the nitrogen was still present as peptides. The amount of nitrogen injected was 5.6 to 8.4 grams a day, or somewhat above the preliminary 2 day excretion

of 5.8 and 4.5 grams during which glucose alone was given; the positive balance varied between 0.55 to 2.1 grams a day. Of special interest was the fact that the peripheral veins were used for the venoclysis for 10 days, when it became necessary to operate and introduce the cannula into the splenic vein, which was used for the last 6 days; during the latter period there was no increase in the degree of positive balance, thus indicating that nitrogen retention was just as good whether hydrolyzed protein entered the portal or systemic blood. The goat died on the 18th day and at autopsy showed no infection, but a massive thrombosis was found extending from the jugular vein to the vena cava. The same authors achieved positive nitrogen balance of 1.6 to 4 grams in 2 dogs for 4 days using the same hydrolysate. Both dogs died on the fifth day. The same year (44) another report appeared in which hydrolyzed meat and casein were injected intravenously in dogs but the experiments were of short duration and not designed to study nitrogen balance. Nevertheless, they represent a milestone in our knowledge of amino-acid metabolism.

No further work was done for over two decades. In 1938 (11) an acid hydolysate of casein was injected intravenously; only when tryptophane and cystine were added thereto did immediate positive balance occur. Three day periods were studied in dogs maintained on a nitrogen-free diet and the amount of nitrogen injected was but slightly more than the output during the preliminary period of 3 days before injection. Conversely, when the 2 amino-acids were omitted, immediately, i.e., by the very next day, the animal went into negative nitrogen balance. Further experiments (12) with this supplemented acid digest of casein showed that nitrogen balance depends upon whether the added tryptophane and cystine were introduced at the same time as the acid hydrolysate or were injected 6 hours later. Curiously enough even such a short delay in the injection of the added amino-acids resulted in a negative nitrogen balance. Thus it would seem that the maintenance of nitrogen balance with intravenous administration is dependent upon, or at least greatly influenced by the simultaneous presence of all of the essential amino-acids in the injected fluid.

An interesting and perhaps important aspect of these two last observations is the rapid time relation of injected nitrogen on the nitrogen output, unlike the delay usually observed when protein is given orally. Thus the step-like increase or decrease in nitrogen output following the addition or withdrawal of protein repeatedly observed by many workers on nitrogen balance with oral administration is apparently not present with intravenous administration of hydrolyzed protein. The fact that much of the theory of protein stores in the body rests upon this behavior of the nitrogen output following orally administered protein gives theoretical significance to these findings. Inasmuch as this step-like phenomenon was not observed and has not been observed by others in subsequent intravenous studies would seem to indicate that the delayed effect of protein given by mouth on nitrogen output has something to do with a lag in digestion and absorption of protein in the gastrointestinal tract and not with the presence of any body store of protein or, as it is often called, "deposit nitrogen." Moreover, the idea that nitrogen balance may be affected by the presentation of all

essential amino-acids to the tissues *at the same time* may also explain some of the delay in the effect of oral administration of protein as compared with intravenous injections.

Several years later from the same laboratory studies (17) were reported following the injection of a protein digest made by the hydrolysis of casein by pork pancreas, a method of preparation which did not destroy tryptophane and which yielded a product containing also the amino-acids present in the pancreatic protein used. While this digest required no amino-acid supplement, unlike the acid hydrolysate, it was incompletely digested in that about 30 per cent of the protein was still in the form of small peptides. In 5 dogs this hydrolysate was given intravenously each day as a 5 per cent solution plus 5 per cent glucose during the fourth week of the study, the first 3 weeks and the fifth week being control periods. Practically no protein was given by mouth during the entire 5 week period; the amount of nitrogen injected during the week of injection averaged 23 grams of nitrogen, or about 3 times the average nitrogen output during the week before and the week after the injection. (There was no increase in nitrogen output during the week following the injection.) During the fourth or injection week the output was 17.8 grams, giving a positive nitrogen balance of 5.2 grams. Of the nitrogen output during this week of injection 7.3 grams, or the control output, would still have been excreted and thus the rest, or 10.5 grams (17.8−7.3) therefore originated from the injected digest, indicating that 54.6 per cent $\left(\frac{23-10.5}{23}\right)$ of the nitrogen supplied was actually retained and utilized by the body. Four similar experiments in which the same amount of hydrolysate was given by mouth showed a 56.6 per cent utilization, thus indicating that maintenance of nitrogen balance was of a similar degree whether the digest was given by mouth or intravenously.

In a study designed to gain information regarding the effect of large injections of protein digests (14) 3½ grams of nitrogen per kilogram of body weight were injected during 24 and 48 hour periods in 6 dogs depleted by a three week non-protein diet. Although positive nitrogen balance was obtained during the period of injection, large amounts of nitrogen appeared in the subsequent days in four experiments in which this amount of nitrogen was injected during 24 hours. However, in the 2 animals in which the injection of the digest combined with glucose was spread over a 48 hour period, positive nitrogen balance was achieved without subsequent loss during the 7 day period following the termination of the injections. This indicated that the injection of 1.7 grams of nitrogen per kgm. per day for 2 days resulted in the same degree of nitrogen retention as the same amount of nitrogen spread over a period of 7 days, and suggested an approximate ceiling of nitrogen utilization, which corresponds to a daily protein intake of about 10 grams per kgm. per day.

Other digests have been investigated. A papain digest of casein was used (32) in 5 dogs and nitrogen balance studied following the injection of a 5 per cent solution thereof with 5 per cent glucose in amounts slightly greater than the basic excretion of nitrogen on a non-protein regime. In 3 dogs depleted by

plasmapheresis the study was made for consecutive 7 day periods. In the first dog a positive nitrogen balance was obtained for 5 out of 7 such periods and for all of the succeeding 6 periods, i.e., for 13 continuous weeks with 2 exceptions. In the second dog 4 similar periods resulted in positive balance of +0.5 and +2.3 grams during 2, and negative balance of −1.7 and −1.5 grams during the other 2, although in 2 subsequent periods in which the digest was injected subcutaneously, a positive balance of +1.7 and +4.3 grams was achieved. In the third dog the digest plus tryptophane and cystine resulted during one period in a slightly negative balance of −0.8 gram. In the remaining 2 animals nitrogen balance studies were carried out on dogs not subjected to plasmapheresis but merely maintained on a low protein diet. During 4 two day periods negative balance was observed as high as −4.6 grams. In a subsequent 2 day period the addition of tryptophane and in another 2 day period the addition of tryptophane and cystine to the digest was still followed by a negative nitrogen balance, although during the following 4 days the same mixture by mouth resulted in a positive balance of as much as +2.1 grams. The same observers also studied another digest of casein which was described as a mixture of acid and alkali hydrolysates. When injected into one dog depleted with plasmapheresis during two 7 day periods a markedly negative nitrogen balance of −10.9 grams and −5.6 grams occurred. Subsequently, during a 7 day period this digest, supplemented by cystine and tryptophane, was injected, resulting in a +3.0 grams positive balance. In 2 dogs not subjected to plasmapheresis this hydrolysate supplemented with cystine and tryptophane yielded one 2 day period of positive nitrogen balance of +0.49 gram, the rest being negative, up to −1.09 grams. In another report (38) from the same laboratory papain digests of casein and beef serum were injected intravenously in dogs maintained on a protein-free diet, but kept anemic by repeated small bleedings. There were 5 observations, in the first two of which beef serum digest was used. In one dog during three 7 day periods a positive nitrogen balance was observed of 20 grams which would be lessened but not eliminated even if both the hemoglobin and plasma removed during this period were subtracted. In the second experiment involving two 7 day periods the positive balance was nearly one gram a day, which would be cut in half if the amount of hemoglobin and plasma removed in bleeding were subtracted. In the next 3 experiments a casein digest was used. In the first of these during two 7 day periods there was a positive balance of +9 grams, which, however, would be turned into a negative balance only if both the hemoglobin and plasma removed during this period were taken into account. This was true also of the next experiment in which during two 7 days periods a positive balance of +16.4 was achieved. In the final experiment a positive balance of +12 grams was achieved during one 7 day period, which would be lessened if both the hemoglobin and plasma removed during the period were subtracted.

Nitrogen balance has been studied following the injection of a gelatin solution (4) which in reality is a protein hydrolysate, inasmuch as it is usually prepared by boiling animal tissues, a process which dissolves and digests the water soluble proteins into particles of varying size; however, it is probable that little of it is

present as amino-acids. In 4 dogs, 6 periods varying between 7 and 15 days showed that in all but one a slight positive balance was achieved. However, the amount of nitrogen injected was 4 to 13 times the amount of nitrogen excreted during the control periods. Moreover, partial data from subsequent experiments indicated that in the period following the injection of gelatin large amounts of nitrogen appeared in the urine, suggesting that the positive nitrogen balance observed during the actual period of injection represented a retention which was subsequently lost. Gelatin, of course, does not contain all of the essential amino-acids and should not, therefore, be able to maintain nitrogen balance.

Various mixtures of pure crystalline amino-acids have also been injected for purposes of protein nutrition. In the first experiments along this line (30) the mixture used contained only essential amino-acids plus glycine and arginine. Seven of the amino-acids used were racemic. The amount of nitrogen injected was the same as in previous periods during which similar mixtures by mouth resulted in positive balance. The dogs were being simultaneously depleted by plasmapheresis but were on a non-protein diet. In the first of two 7 day periods a negative balance of −5.4 grams was observed but is probably explained by carry-over of the effect of a deficiency in threonine in the mixture injected during the preceding period; in the second 7 day period, moreover, a positive balance of +0.5 gram was achieved. In a subsequent paper by the same authors (38) a similar mixture of amino-acids was injected intravenously for 7 days in a dog kept on a non-protein diet, but bled frequently to produce anemia. A positive balance of +11.6 grams was observed during the period not calculating the amount of nitrogen removed as hemoglobin and plasma which, however, totalled but 3.7 grams. In another laboratory (16) a mixture containing only the 9 essential amino-acids were injected in dogs taking only sugar by mouth, which showed that nitrogen balance could be achieved thereby during 3 day periods following the injection of an amount of nitrogen which was only slightly more than the amount excreted during the control periods. Of special interest was the observation that the omission of histidine from such a complete mixture was still followed by positive nitrogen balance for a period of 3 days, but that a negative balance followed during 2 subsequent 3 day periods, suggesting that histidine is relatively stable and, unlike tryptophane, requires a longer time before its absence is manifested in nitrogen balance experiments. This lag in the production of negative balance was also observed in the experiments on the intravenous injection of gelatin just mentioned, and in one of the reports on plasma transfusions. As will be discussed later, these observations add further support to the idea that important time factors must be considered in all studies on nitrogen balance.

Observations in the human. Though first used but 6 years ago, many observations have already been made on the maintenance of nitrogen balance in the human following the intravenous injection of various hydrolysates of protein. This discussion will be confined to studies in which the injected digest formed the *sole source of nitrogen*. No observations will be included in which these injections were combined with the oral administration of protein food because of obvious difficulty in evaluating the relative influence of the two sources of nitrogen on nitrogen balance.

It was only in 1938 that hydrolyzed protein was injected intravenously into the human for the first time (18). Positive nitrogen balance was achieved with an acid hydrolysate of casein supplemented by tryptophane and cystine. Eight cases were described, although detailed data were mentioned in only 2 instances. One, a 67 year old woman, unable to take anything by mouth because of an inoperable carcinoma of the stomach, was maintained with saline and glucose with a level of nitrogen output in the urine of 4 grams a day; on 3 successive days positive balance followed the addition of 4.5 grams of nitrogen as the protein digest. In another case, a 69 year old woman with severe septicemia, in coma, exhibiting nutritional edema, 5.16 grams of nitrogen as the casein digest was added to the intravenous drip of saline and glucose for 3 days. The urinary nitrogen, previously 4 grams a day, increased to 4.5 grams a day, thus producing a positive balance of +0.66 gram a day for the 3 days. In a subsequent report (13) further clinical experiences were described in which an enzymic hydrolysate of casein was used, requiring no addition of supplementary amino-acids. This preparation was made by the digestion of casein with pork pancreas and therefore contained the amino-acids present in the pancreas protein as well as in the casein. This digest, moreover, unlike the acid hydrolysate which contained no peptides, was incompletely broken down so that about 30 per cent of the nitrogen was present in the form of small peptides. The term Amigen has been applied to this last preparation by the manufacturers and for simplicity will be used in the following discussion. This report described the use of Amigen in 35 patients, of which 8 cases were described in detail. During a preliminary period a solution of 5 per cent glucose in saline was injected and compared with subsequent periods in which the hydrolyzed protein was merely added to the same amount of glucose. In many of the cases there was a second control period after the injections. Positive nitrogen balance was achieved in 4 of the 8 cases, partially so in 2 and incompletely in 2. The daily amount of nitrogen given did not exceed 9.6 grams, which was somewhat more than the output during the preliminary period in the first 4 cases mentioned, but considerably less than the output in the second 4 cases which were more seriously ill patients and, probably because of this, showed an unusually high nitrogen loss. Failure to achieve consistent nitrogen balance in the last 4 cases may be attributed to the fact that an insufficient amount of nitrogen was injected. There is little evidence in these experiments that the introduction of the digested protein of itself led to a significant increase in the nitrogen output. For example, in the first case, a relatively normal individual, the nitrogen output in 3 preliminary control days averaged about 5.5 grams; for 12 subsequent days, during which 7.2 grams of nitrogen was added to the glucose, the positive balance varied between 1 and 3 grams a day. In the second case the output during the control period was somewhat higher, averaging about 6.5 grams a day, yet positive balance was also achieved although it did not exceed one gram a day.

Other observers used the same hydrolysate in 30 postoperative patients (20) with similar results. The solution contained 3 per cent Amigen and 7 per cent glucose and was injected for an average of 7 days, during which no food was ingested. The amount of nitrogen injected was 7.2 grams per day. Details of

nitrogen balance were described in 2 patients. In one, following a simple appendectomy without complications, positive nitrogen balance of +1.4 to +3.1 grams per day or a total of +14.6 grams during 6 days was achieved. In the second patient, who had peritonitis, the same amount of Amigen did not result in positive balance; indeed there was a negative balance of −2.1 to −12.9 grams a day. These findings were stated to be characteristic of the other cases, i.e., positive nitrogen balance could be obtained with this dose of Amigen nitrogen only when the patient was excreting less than this amount, but not in the sicker patients with higher losses in the urine, which in one case amounted to 25 grams of nitrogen in one day. Another observer (9), using the same digest, was able to produce positive nitrogen balance in a 57 year old male who was studied for 18 days during which nothing was taken by mouth; data during 13 days in which urine collections were complete showed that the injection of Amigen in amounts varying between 6 and 12 grams per day with added glucose and a fat emulsion resulted in a positive nitrogen balance of between +0.5 and +5.2 grams a day in all but 2 days. During these 2 days the negative balance was −1.8 and −1.6 grams of nitrogen. In general the highest positive balance was observed when the largest dose, i.e., when 12 grams of nitrogen, was injected. In a subsequent report (3) this same observer studied an adult female for 6 days during which the patient received nothing by mouth, the injections containing 18 grams of nitrogen as Amigen plus 300 grams of glucose. A positive balance of 23.3 grams of nitrogen was observed during this 6 day period or nearly 4 grams a day. In another more detailed study (5) from the same clinic data are described on 9 patients, all of them operated upon and showing huge losses of nitrogen in the urine, undoubtedly as a result of their disease. A positive nitrogen balance in general was not consistently achieved with the amounts injected, probably because of the large loss of nitrogen in the urine, although in one patient for a period of 10 days during which 18 grams of nitrogen was injected each day, a positive nitrogen balance of +48.84 grams was observed, or nearly +5 grams a day.

Nitrogen balance studies (26) following the injection of Amigen solution as the sole source of nitrogen were reported from still another clinic, the details of 2 cases being described. In the first case a patient with squamous carcinoma of the lower end of the esophagus was first studied for 6 days before operation. Following a control period of 3 days in which no nitrogen was injected, positive nitrogen balance was achieved in the next 2 days, during each of which 12 grams of nitrogen were administered. On the sixth day 6 grams of nitrogen were administered with a negative nitrogen balance. At operation the lower end of the esophagus and cardia of the stomach were resected, followed by a primary anastomosis. On the second day after operation, during which the patient received only saline and glucose, 15.6 grams of nitrogen appeared in the urine. On the next day 9 grams of nitrogen as Amigen were injected with a positive balance of +1.7 grams. On 4 subsequent days the nitrogen balance was negative (−0.1 to −5.1 grams a day) on an intake of Amigen of but 6 grams. On 2 subsequent days during which no nitrogen was injected the output was 10.13 grams per day.

In the second, a non-operative case, between 6 and 12 grams of Amigen nitrogen were administered each day as the sole source of nitrogen for 8 days. During this period there was a total positive nitrogen balance of +6.7 grams, or an average of nearly a gram a day.

In a series of reports in infants, Amigen was injected intravenously for varying periods of time as the sole source of nitrogen. In the first study (42) six 2 day periods were studied on 4 infants and one older child, a positive nitrogen balance being obtained in all but one case, the degree of which was calculated as varying between 3 and 46 per cent of the amount injected. These figures were then compared with similar periods in which the same amounts of nitrogen were ingested as a milk formula and the degree of retention was practically the same. In a second study (41) the observations are of interest even though not designed to give information as to nitrogen balance. The nitrogen excretion in the urine was compared in infants during five separate 24 hour periods in which Amigen was injected with two 24 and one 12 hour period in which a mixture of crystalline amino-acids made up to contain the estimated composition of casein. Although nearly one-fourth of the amino-acids of the crystalline amino-acid mixture was in the unnatural form, the excretion of nitrogen in the 2 groups of observations was entirely similar. In a third and more detailed study (40), 20 male infants were given Amigen intravenously and compared with control periods in which only glucose and saline were injected. Positive nitrogen balance was achieved when the nitrogen intake exceeded 0.31 gram per kgm. per day, but was greatest when the intake was 0.92 gram per kgm. per day, the latter amount producing a positive balance of +0.50 gram per kgm. per day, i.e., 54 per cent of the intake. These figures may be calculated in another way by using as a baseline the control output of nitrogen which is given as −0.15 gram per kgm. per day. In the first series of observations during which 0.50 gram per kgm. per day was given, the output was 0.34 gram. Now 0.15 gram may be subtracted from 0.34 gram as representing the control output, which gives 0.19 gram as representing that part of the output originating from the injected digest, which when subtracted from 0.50 gives 0.31 as the nitrogen actually retained and this is 62 per cent of the amount injected. Similarly, at the higher level of injection of 0.92 gram during which the output was 0.42 gram, a similar figure of 70 per cent retention may be obtained. It will be also observed that twice as much nitrogen had to be injected as was excreted during the control period in order to achieve nitrogen balance.

An acid hydrolysate of casein has been used in other observations. Tryptophane was added to make the mixture complete, inasmuch as such hydrolysis was followed by destruction of this amino-acid. Earlier observations with a similar hydrolysate have already been mentioned (18). In one adult (1) such a hydrolysate was used as the sole source of nitrogen after operation for intestinal obstruction, but nitrogen balance was not achieved. During a period of 8 days there was a daily negative balance of −3.58 grams with an intake of 5 grams, which was greater than the output of 3.96 grams during the control period of 4 days during which glucose and saline alone were injected. Very large amounts

of creatin were excreted in the urine during the period in which the digest was injected. Successful achievement of positive balance, however, was achieved with the same hydrolysate as the sole source of nitrogen by another observer (34) in a patient operated on for pelvic abscess. There was a preliminary control period of 5 days in which there was no nitrogen intake. Following this was a 6 day period in which the nitrogen intake consisted of transfusions of blood as well as injection of the casein digest, and positive balance was observed. However, during a third 5 day period the digest formed the sole source of nitrogen; 12 to 18 grams a day were injected intravenously and daily positive balance of +1.1 to +4.9 grams was observed.

COMMENT. Maintenance of nitrogen balance, as shown by the studies here summarized, can be achieved both with plasma transfusions and with the intravenous injection of mixtures of amino-acids either as pure crystals or as hydrolyzed protein. In the case of plasma, compatibility is a prerequisite for there is sufficient evidence to show that even plasma from the same species will lead to negative balance if there is an untoward reaction. Even in the absence of such reaction, there is some evidence that positive balance may require donor plasma which is of the same type as the recipient, although much further study is needed. The influence of the added anticoagulant may also prove of significance.

In the case of amino-acid mixtures, the presence of all of the essential ones in the injected solution is a clear prerequisite for maintenance of nitrogen balance at least for periods of more than 3 days. With injections of hydrolyzed protein, failure to maintain balance with some preparations is undoubtedly also referable to a deficiency in one or more of the essential amino-acids. The influence of untoward reactions following the intravenous administration of hydrolyzed protein is probably the same as in the case of plasma, although no observations are available.

In several of the studies there are indications that glucose should be added to the solutions of hydrolyzed protein in order to achieve nitrogen balance more effectively. While the importance of glucose seems obvious on theoretical grounds, there is insufficient data to show just how important this consideration may be. It is hoped that further studies will be made with varying proportions of glucose in order to show whether glucose is really required, and if so, what the optimum amount may be.

Further consideration of the data reviewed herein invites 2 comparisons regarding maintenance of nitrogen balance with intravenous injections of nitrogenous nourishment. First are differences between this method and the oral one; second are differences between the intravenous injection of whole protein as plasma transfusions and the injection of the building stones of protein as hydrolyzed protein or mixtures of amino-acids. Inasmuch as protein nourishment normally enters the blood stream as amino-acids or small peptides, it is obvious that a plasma transfusion introduces entirely new metabolic problems as compared with the ingestion of the same plasma proteins by mouth. In other words, the introduction of whole protein molecules directly into the blood stream has no physiological counterpart in the normal mechanisms of nutrition. That the

injected protein leaves the blood stream rapidly and to a great extent seems clear. On theoretical grounds, however, it is difficult to predict whether the injected plasma protein which leaves the blood stream is utilized as such by body tissue, whether it is broken down to smaller units, even to amino-acids by cellular proteases or indeed whether it simply undergoes an overhauling process by the interchange of free amino-acid groups without hydrolysis before being used elsewhere. The evidence herein reported, though limited, does indicate that nitrogen balance is more readily achieved by the intravenous injection of plasma protein than by its oral ingestion, thus indicating more efficient utilization in the former case. On the other hand, longer observations are probably necessary in view of the fact that plasma protein breakdown is a slow process. Observations with isotopic nitrogen indicate that the half life of plasma protein is about 14 days. Nitrogen balance studies following plasma transfusions, therefore, should probably be continued for 2 or more weeks in order to determine whether the nitrogen injected is really retained permanently or whether there is merely a delay in its excretion. A time factor is also important in the behavior of hydrolyzed protein injected intravenously, as already discussed. It is suggested, moreover, that growth experiments be also carried out with plasma protein inasmuch as important evidence of the amino-acid composition of plasma protein can be obtained in this way, which may be of value in correlating data on nitrogen balance.

The above mentioned differences between the oral and intravenous administration of plasma do not apply to hydrolyzed protein inasmuch as all protein food is normally hydrolyzed before absorption. Thus the injection of appropriately hydrolyzed protein or amino-acid mixtures introduces no new or unusual physiological mechanism except for the fact that the material enters the systemic rather than the portal circulation. Although the liver is probably the most important organ in the metabolism of amino-acids, other tissues, of course, do play an important part. While there is no theoretical reason why there should be any difference in utilization between amino-acids entering the portal vein and the systemic veins, the rather limited evidence, as reviewed herein, does, with one or two exceptions, point to the similarity in the nitrogen balance produced by the two modes of administration. An analogy may be drawn by citing the fact that the intravenous injection of glucose leads to glycogen storage in the liver as well as does oral administration.

The inclusion of protein nutrition in parenteral injections now extensively used for patients unable to take anything by mouth is but a natural extension of this mode of therapy which for years has been confined to the administration of saline and glucose solutions plus certain vitamins. It is probable that this method of treatment will be used more and more as the importance of preventing or correcting protein deficiencies in this way becomes more generally realized. Acute starvation in patients unable to eat requires the parenteral administration of a more or less complete diet and this, of course, is impossible unless nitrogenous nourishment is included. Much more investigation must be done before the metabolism of intravenously injected protein nourishment is fully understood,

particularly regarding the injection of the larger molecules and especially the whole protein molecules in a plasma transfusion.

SUMMARY

Review of the literature through 1943 reveals sufficient evidence of the fact that nitrogen balance may be achieved both in animals and man by the intravenous administration of compatible plasma as the sole source of nitrogen. Nitrogen balance can similarly be maintained by the intravenous injection of solutions containing mixtures of all of the essential amino-acids or certain protein hydrolysates.

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THE FUNCTIONAL ORGANIZATION OF THE CEREBRAL CORTEX

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Functional organization, which defines the temporal course of activity in any nervous mechanism, depends upon both physico-chemical reactions of constituents and their anatomical relations. Since reactions of all neurons are similar, it is frequently possible to deduce anatomy from observed activity or to predict activity from known anatomy.

GENERAL ORGANIZATION. From a single enlargement of the neural tube, the prosencephalon, arise the cortex cerebri and the corpus striatum anteriorly and the thalamus posteriorly. At a comparatively early stage of development antéro-posterior and postero-anterior connections are established. They serve to relate certain portions of the cortex and basal ganglia (1, 2) to particular thalamic nuclei—which relay signals thither and receive them thence. Also at an early stage, there emerge from the cortex axons which, passing ventrally, make their way to efferent structures. Thus, functionally, these forebrain structures constitute a bridge of internuncials which in lower animals is but one of many. As the cortex evolves, the other bridges either disappear or become unable to mediate all sense modalities. This development correlates with increasing anatomic differentiation of the thalamic nuclei, which have few or no internuncials and, with few and small exceptions, no descending connections. Hence, as the forebrain structures become necessary for afferents of a given sense modality to affect efferent systems, the cortical or striatal link also becomes necessary, and that in two ways. It is both the internuncial system co-ordinating activity within and among the thalamic nuclei and the only significant path thence to the efferent system.

The obvious paralyses resulting from injuries of certain cortical regions and the motor responses to their electrical stimulation have led us to call them "motor" or "electrically excitable"; but today we know that most, if not all, regions of the cortex send axons to structures whose excitation affects the contraction of striated muscle, and that all parts of the cortex are electrically excitable. Today we would not use these properties in subdividing the cortex. The thalamic nuclear connections are discrete and serve to define "cortical sectors" consisting of cytoarchitectonically recognizable areas which preserve their topological relations—i.e., those which are not altered by stretching or folding—presumably because the formation of these thalamo-cortical connections is causally related to the local differentiation of the cortex, and because they remain despite the growth and deformation of the continuous mantle as it evolves into the convoluted cortex. Moreover, certain thalamic nuclei relay, in an orderly fashion, impulses consequent upon specific sensory excitation, and the corresponding cortical sectors exhibit this specificity and orderliness which help to define the principal rôle played by any sector and to analyze its spatial detail

in relation to that of the sense organs represented upon it. Even with respect to the associational structures which become disproportionately larger in the primate cortex and have thalamic relations with nuclear structures other than primary sensory relays—namely, parts of elaborate re-entrant circuits—the same clarification of function occurs for the same reason. Thus by conscientiously ignoring the obvious deformations, gyri and sulci, save as dubious landmarks in a particular cortex, it is possible to discuss in general the function of sectors and their constituent areas. Although the function of specific subdivisions of the sectors in other ways is various in various species, the functional homologies of these other subdivisions, insofar as they have corresponding thalamic correlates, are surprisingly orderly. This makes it possible to transfer to the cortex of a given species conclusions drawn from experiments upon other species. Since we are primates and most work has been done on the monkey, *Macaca mulatta*, it seems best to use figures of his cortex as a frame of reference for what follows.

A cursory glance at the gross anatomy of any mammalian brain shows that the cortex on which a thalamic nucleus projects is many times larger than that nucleus, and detailed microscopy reveals that there is great overlapping of the projections. A single thalamic nuclear element thus projects to a vastly greater cortical area. As will appear clearly later, the connections in the reverse direction spread in a similar fashion. Thus the area of cortex to which a single thalamic neuron projects serves to relate it to a large portion of its own nucleus. On this account the nucleus and its cortical sector tend to behave concertedly in a rhythm determined by the time of transit around their reentrant path. At any part of the circuit the resultant waves affect the threshold to incoming stimuli and may be initiated or interrupted by them. Whether these waves be the envelopes of nervous impulses or merely of polarization and depolarization, they are prevented by any lesion which prevents corticothalamic reverberation. Residual activity lacks their characteristic form and frequency. Other deeper structures may contribute to the background of the thalamic excitation, and their contributions may be necessary, but the "spontaneous" electrical activity will persist in a small island of cortex when all the rest of the cortex of that sector has been removed (3)—a finding which explains why diffuse pickup fails to reveal local cortical destruction.

It goes almost without saying that these same circuits serve as internuncials to relate the activities of diverse portions of the cortical sector. That these cortico-thalamo-cortical circuits play a fundamental role in determining the organization of cortical activity is attested by the synchronizing of disturbances at two parts of the same cortical sector after severance of all direct cortico-cortical connections. A similar but more complicated re-entrant path arising from cortical efferent connections serves a similar function for even larger subdivisions of the cortex. Thus, cortico-ponto-cerebello-thalamo-cortical circuits, arising from frontal, parietal and temporal regions and playing upon the anterior portions of the ventral thalamic nuclei, affect the activity of the anterior portion of the central, or sensory, sector.

This general character of overlapping projection extends to the control of

motion and makes possible use of parts whose focal cortical representation has been destroyed. If the lesion be made early enough (4), although it be large, the loss of motion is far from total and investigation of the cortex by electrical stimulation indicates that other portions of the same cortical sector have far more control of the affected muscles than can be demonstrated in the intact cortex or following a similar lesion in the adult. However, no sector has ever been shown to assume a function to which it did not contribute prior to the lesion. The corpus striatum acts in parallel with the cortex and hence complete decortication leaves a well established thalamo-striatal prosencephalic bridge of internuncials, over which afferent are connected to efferent systems so that even in primates certain motor activities may still be mediated in response to stimulation of some sense organs.

Relatively diffuse but overlapping projections upon the cortex where summation results in focal excitation make possible detailed discrimination, exceeding in sharpness of outline the distribution of excitation of sense organs (5), while interconnection of these cortical foci (6) via recursive connection through deeper structures and via direct cortico-cortical paths permits the activity of each focus at any instant to be affected by the antecedent and approximately contemporaneous activity of all other foci. The resultant efferent discharges are thus the unified consequence of the totality of these related cortical focal activities. Since activities of all parts of the cortex are related by recursive activities through deeper structures, severance of purely cortico-cortical connections can be expected to destroy only the most nearly contemporaneous relations of cortical activities at the separated foci, and so to affect adversely interpretations dependent upon them. As yet we have no behavioral test whereby we might hope to detect them. This is borne out by the lack of any discoverable loss of ability following severance of the entire corpus callosum, for this is the largest single bundle of such connections and the only one severable without any cortical destruction (7, 8).

On the other hand, overactivity of cortico-cortical connections produces a spread of disturbances over the cortex along these lines of communication. This is all too familiar in the spread of the cortical activity responsible for the march of the Jacksonian convulsion, and today there is clear evidence that for this spread of disturbance direct cortico-cortical connections are necessary (9) and sufficient (10).

COMMON PROPERTIES. Before specifying particular thalamo-cortical projections or cortico-cortical connections, common properties should be considered, for these determine in large measure the significance of the particulars, and the interpretation of electrical or other records of cortical activity and its consequences.

Impulses entering from the subjacent white matter reach all parts of the cortex via axons which ascend to its middle layer, where they branch widely and ramify as they ascend. This axonal activity is manifest electrically by the appearance of a transient voltage in which the depth becomes negative and the surface positive to any unaffected area. This phenomenon is not prevented by rendering the neurons of the recipient cortex unresponsive by local treatment with

drugs. Having ramified, the incoming disturbance excites neurons of the superficial layers, producing a local surface negativity. Under light barbiturate anesthesia this may be all that happens. Under chloralose anesthesia, and sometimes under very light barbiturate anesthesia, this surface-negative wave is propagated slowly—say 20 cm. per second—in the feltwork of the cortex, dying out (11) as it travels. Under appropriate conditions it can be elicited by weak electrical stimulation of these layers, and has been called the "superficial response." Under extremely light anesthesia, or when augmented by dilute solutions of excitants such as strychnine or metrazol, the surface-negative, or superficial, response is followed by a surface-positive, or deep, response associated with discharge of cells in the deeper layers of the cortex, whence originate axons to remote portions of the cortex, as well as those to subcortical structures. These waves can be elicited regularly by stronger electrical stimulation and are conducted with axonal velocities. There are, then, two types of purely cortico-cortical connections relating foci, at each of which arriving disturbances ascend to the middle layer, ramify upward and descend to leave the cortex. The first is the slow, superficial, surface-negative, presumably multineuronal, or multisynaptic, intracortical type, exaggeratedly active under chloralose; the second is the rapid, deep, surface-positive, presumably axonal, intercortical type, best seen under dial. The intracortical are as diffuse as the feltwork of the cortex, whereas the intercortical are highly specific and restricted in their distribution. Under chloralose both play significant rôles in determining the spread of electrical after-discharge; under dial, only the latter (11). These types differ also in their effect upon incoming stimuli, for impulses arriving during surface negativity fail to excite, whereas those arriving during surface positivity are facilitated,—a relation which throws some light on the effect that alpha rhythm has upon response to stimulation of the eye or of the lateral geniculate, and on the driving of alpha rhythm by visual stimulation (12, 13). Taken in conjunction with inhibition at a synapse in the case of the two neuron arc of the spinal cord (14), the relation of transcortical potentials to responsiveness to incoming stimuli suggests that inhibition is to be expected when the apical dendrite of any cell is in a region negative to its axon hillock, and facilitation when polarization is the reverse.

Weak electrical stimulation of the superficial layers of the cortex, if repeated at appropriate intervals, elicits a depth response which increases to a certain maximum determined by the duration and voltage of the stimuli. This facilitation occurs at all cortical foci and is manifest in motor response from many areas. It is presumably due to spatial summation operative over relay paths and reverberating chains of internuncials. That it has a maximum dependent upon other parameters of stimulation is presumably due to the same limitation of responding efferent cells that produces occlusion in the case of spinal reflexes, with this difference: that in the case of spinal reflexes all cells of the pool may be excited, whereas in the case of the cortex the number that may be called into play depends upon the voltage and duration of the stimulus. Increase in voltage can always spread the stimulus to more remote portions of the pool, and increase

in duration of pulse will, within limits, always fire a larger proportion of the neuronal pool reached by a given voltage. Finally, repetitive activity at frequencies of 20 or more per second eventually produces a rise in threshold of the responding neurons, so that if the stimulation be continued the response declines or even disappears. This rise in threshold is presumably the chief factor for the extinction of motor response to cortical stimulation, and undoubtedly is of paramount importance in all problems of fatigue. It is associated with the development of a polarization of the correct sign to enhance the effect, and perhaps to extend it to other neurons in the vicinity. In those cases in which stimulation of any cortical area is pushed so far as to initiate self-sustained activity—called after-discharge—another factor becomes significant, for the oxygen supply, though it may increase, is locally inadequate for the demand,

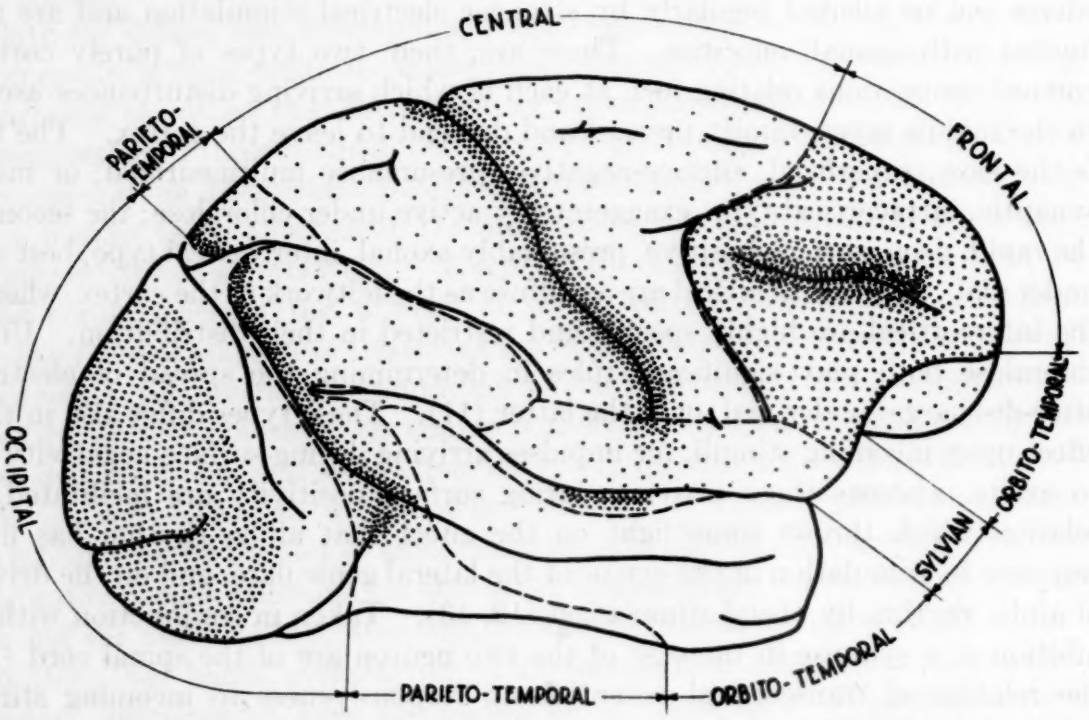

Fig. 1. *Macaca mulatta*. Thalamic projections to cerebral cortex and division into regions described in text.

the oxygen tension falls (15) and lactic acid is produced (16). As the region becomes acid its threshold rises and eventually the self-sustained activity ceases. It may have spread over cortico-cortical connections to other regions of the cortex, but those it leaves behind are inactive and unresponsive. These are the aspects of functional organization which all parts of the cortex have in common.

CORTICAL SECTORS AND REGIONS DISTINGUISHED. Thanks in no small measure to Walker's (17) and Le Gros Clark's (18) studies of retrograde degeneration of thalamic nuclei following destruction of various cortical regions, the "sectors" of the monkey's cortex defined by their thalamo-cortical connections are relatively well known. They are schematized roughly in figure 1 which also indicates the density of thalamo-cortical projection by the density of stippling. It is apparent that certain regions are dominated by these projections which define the *cortical sectors*.

The projection from the lateral geniculate body in primates is sharply limited to area 17, the area striata, which is surrounded by area 18, and this, in turn, by area 19, known respectively as parastriate and preoccipital. The occipital region, constituted of these three areas, has a close functional organization subserving vision.

Similarly, there is a parieto-temporal region dominated by projections from the medial pulvinar nucleus to the posterior parietal cortex and by the inferior pulvinar nucleus to the posterior temporal cortex. The whole region plays an important rôle in associative processes.

The largest single region is the central or "sensory" region, which receives impulses from the lateral thalamic nuclei. Those anterior to the central sulcus come from the ventrolateral, and those posterior from the posterolateral. The former are most dense in area 4, the latter in area 3, but as all parts of this region receive some, this sector is coextensive with this region.

Anterior to it is the frontal region whose thalamic projections arise from the dorso-medial nucleus and are most dense in a relatively small area which Walker has called area 46 for cytoarchitectural reasons. This region is granular cortex and includes all cortex anterior to the central sector on the lateral aspect, with the possible exception of area 8 which will be included in it to simplify description. On the medial side it extends just across the sulcus callosomarginalis. It does not include the opercular areas or any orbital areas proper. The latter belong to the orbitotemporal region.

The former, opercular, areas belong to the sylvian region which is dominated by the projection of the medial geniculate body to area 41 and with less density to area 42, known respectively as the primary and secondary auditory cortex and lying on the supratemporal plane, which is therefore referred to as the sector of the medial geniculate body.

The sector of the anterior thalamic nucleus lies on the posterior portion of the medial aspect of the cortex and is coextensive with the posterior limbic region, consisting of areas 23 and a small retrosplenial area which is probably best called area 29.

The thalamic projections to the remainder of the iso-cortex are unknown. This includes the anterior limbic region, or area 24, and the large orbito-temporal region which includes all the areas confined to the orbital surface of the frontal pole and the tip and greater part of the lateral aspect of the temporal lobe. These are here regarded as one region, not because there are no known thalamic projections to them but because they are related in activity. Of the remaining isocortical area, 25—and of the allocortical areas 28 and 36—we lack the knowledge to assign them to any region.

CORTICAL SECTIONS AND REGIONS DESCRIBED. Among the regions of the isocortex there are three having sectors whose activity represents sensory excitation mediated by thalamic nuclei in such a manner as to preserve topological organization—namely, the occipital, the central and the sylvian. These have been known for a relatively long time because lesions of these areas produced loss of the corresponding sensation or because excitation of the area, either in the aura of convulsive disorders or in the waking subject stimulated at operation,

produced the corresponding sensation. More recently they have been investigated by stimulation of the receptor (19) and observation of the induced electrical disturbance.

OCCIPITAL REGION. By fixing the eye and flashing a small light in known relation to the point of fixation, while recording from electrodes in fixed positions on the occipital lobe, the visual field has been plotted on the cortex. In the primate, off and on responses are sharply restricted (20) to area 17. The point of fixation is represented on its lateral or anterior margin and only one half of the field falls on one hemisphere. Small angular displacements from the fixation point record at relatively large distances from its representation, but as the light is presented at angles increasing in equal steps the displacement per step is less on the cortex. The horizontal meridian of the visual field maps almost horizontally backward from a point about the height of the junction of the first temporal sulcus and the sylvian fissure, in the direction of the superior ramus of the calcarine fissure. The visual field represented is contralateral and inverted. It is on this area to which each retinal point projects to an appreciable circle that summation results in disproportionately sharp differentiation, so that fine eye movements combined with this summation result in discriminative ability far exceeding the analysis of the stimulus of which the retina itself is capable (5).

To investigate the connections of various cortical foci, it is possible to stimulate at one focus and record induced electrical activity propagated to remote foci. Electrical and chemical stimuli work, but the former are more difficult to control, for the current spreads to deeper structures where it excites subjacent axons, while it sets up antidromic impulses even in the gray matter. These effects are minimized by using relatively long pulses at relatively low voltages, but they cannot be entirely excluded even under optimal conditions. Hence the bulk of the work has been done with chemical stimulation. If one places a square millimeter of filter paper soaked in a saturated solution of strychnine sulfate anywhere on the cortex, the cells of the subjacent cortex send out impulses in unison. The resultant spike-like record of the transient voltage can be obtained from any point to which a sufficient number of affected cells send their axons. For brevity, we say that the strychninized focus "fires" these other points. At present there is reason to believe that strychnine poisons an acetylcholine esterase (21) and that this permits acetylcholine to accumulate, whence the cells are discharged so easily that the electrical impulse of any cell excites all the strychninized cells. In any case strychnine acts only where synapses are present on nerve cells and produces disturbances propagated only in the ordinary direction of conduction.

Local strychninization within area 17 produces these spikes but they are not propagated to points of area 17 more than about 1 mm. from the strychninized focus. At the same time there appear large spikes in the record of that portion of area 18 which is nearest to the focus. We say, therefore, that area 17 fires itself only "locally," and fires area 18. Similar strychninization within area 18 fires an adjacent sector of area 17, fires almost all parts of area 18 of that hemisphere and the corresponding point in area 18 of the opposite hemisphere—and

fires much of area 19 ipsilaterally. Thus area 17, which receives impulses in a relatively discrete fashion, keeps them so within itself but relays them to a larger fraction of area 18, which feeds excitation back into the originating segment of 17 and forward into area 19. With a relatively light Dial narcosis one frequently obtains, following both "on" and "off" effects produced by a bright light, a series of smooth, somewhat sinusoidal waves. The "on" and "off" effects are limited to area 17, but the consequent waves spread forward across area 18 to die out in area 19. Moreover, electrical stimulation of any of these areas under appropriate conditions causes deviation of the eyes in a direction and manner dependent upon the exact site of stimulation. With ordinary 60 cycle currents one obtains from stimulation of any point in area 17 a sustained deviation which would bring foveal vision to bear on the corresponding point in the visual field (22). This form of stimulation is less effective in area 18 and the response is transient. Using pulses of rapid rising and slow falling phases, and a frequency up to about 40 per second, responses are greater and more prolonged but still not sustained. Their direction corresponds to that of the sector of area 17 to which the selected focus of 18 corresponds. Even slight stimulation of area 19 produces a relaxation of any existing muscular contraction and, in the vicinity of the intraparietal sulcus, pupillary dilatation. Moreover, stimulation of 19 will hold in abeyance the motor after-discharge, but not the cortical electrical after-discharge, set up by antecedent stimulation of other cortical foci. Finally, stimulation of this region causes a suppression of motor response to stimulation of any motor focus. This phenomenon has a latency of about four minutes, lasts from three to thirty minutes, and connot be repeated with any certainty sooner than forty-five minutes from the time of the stimulation that induced it. For brevity all three of these induced motor inactivities will be referred to as "suppression of motor functions." There is much evidence that they do not depend on any direct or indirect cortico-cortical connections or on cortico-striatal connections, but there is no certainty as to what descending paths mediate them. In these three suppressions cortex (23) and corpus striatum (24) act in parallel fashion.

Strychninization of area 19 causes only local firing and, with a variable latency of many minutes, a diminution or complete disappearance of spontaneous electrical activity which recedes slowly across the cortex, requiring twenty to forty minutes to reach the most remote regions. Before it has reached them activity returns to the nearer regions, at first in the form of "spindles" and later in its original form.

This suppression of electrical activity, like the suppression of motor function, can be elicited from several areas—which are therefore called suppressor areas. All such areas, except 19, have been shown to project to the nucleus caudatus, and for the suppression of electrical activity by them the nucleus caudatus has been shown to be necessary. Its strychninization produces large, long voltages in the thalamic nuclei which may well break up their cortical reverberations. These presumably are not simple axonal impulses from the nucleus caudatus because of time relations and wave form. They resemble the belated and extended post-synaptic consequences of the relatively synchronous discharges of

the pre-synaptic axons. In any case, when they appear in a thalamic nucleus the "spontaneous" activity of the corresponding cortical sector disappears. Until recently no trace of cortico-striatal activity could be discovered arising in area 19, but recently we have seen bumps in records from the nucleus caudatus which were definitely synchronous with strychnine spikes in area 19. Thus it, like all other suppressor areas, probably does produce its suppression of electrical activity via the nucleus caudatus. We will consider the extrinsic cortico-cortical connections of this region only after the intrinsic regional connections of other regions have been considered.

CENTRAL REGION. The central sector has been mapped for its most direct sensory projection by punctate stimulation exciting single points of the body surface (25) or single hairs, and locating the induced surface-positive waves on the cerebral cortex. The excited points project principally to the posterior lip of the central sulcus. The representation of the extremities occupies a disproportionately large portion of the recipient cortex. The leg is represented above a line joining the superior precentral sulcus to the superior postcentral sulcus. The arm is represented below this line to another joining the spur of the arcuate sulcus to the tip of the intraparietal sulcus. Although there is much overlapping in each of these subdivisions, the following general statements can be made. The leg subdivision begins at the sulcus callosomarginalis, which represents the tail, coccygeal segments 4 through 3; then sacral segments 3 through 1; then lumbar segments 7, 6, 5, 4; and finally, lumbar 3 through 1, overlapped by thoracic segments 12 through 1 which appear at the line of junction of the arm and leg subdivisions. Then come the cervical segments (again overlapping) in the order 2, 3, 4, 5, 6, 7, and last 8, which occupies almost the lower half of the arm subdivision. The face area below is more complicated, for the mandibular division of the 5th nerve projects above, behind and below the other two, of which the ophthalmic is above and the maxillary below. As the thalamic nuclei mediating sensibility from face, arm and leg are anatomically distinct and have been identified functionally, it is possible to subdivide the central sector along the lines described above, and to delimit these subdivisions in all primates. The anterior lip of the central sulcus receives the densest projection of the anterior portions of these same thalamic nuclei; and the motor responses elicitable by its electrical stimulation are of the somatic parts projecting to the corresponding post-central area, except at the junctions of the subdivisions where the cortex of the anterior lip is most deeply infolded. Thus the subdivision obtains for efferent as well as for afferent connections. Actually, alteration of somatic muscular contraction can be obtained from all parts of this sector under appropriate conditions, and sensory phenomena can be elicited by strychninization of any part of it, and all exhibit the same subdivisions for leg, arm and face. The conditions for obtaining motor response from points far posterior to the precentral lip are a hyperactivity of deeper structures, frequently appearing under light barbiturate anesthesia, and stimulating pulses having an abrupt rising and a 5 to 10 millisecond falling phase at a frequency of 1 to even 10 or more per second, delivered to a cortex already excited at a focus from which motor re-

sponses have been easily evoked. The response is then that elicited from the original focus. This form of so-called secondary facilitation, at least in certain instances, depends upon axons descending from both areas to the same deeper structures, rather than upon cortico-cortical connections (26). There is another form of secondary facilitation most easily demonstrated from points in the anterior portion of the central sector to points of the anterior lip of the central sulcus. Antecedent stimulation of the anterior point makes it possible to obtain the original response to excitation of the posterior point with a weaker stimulus, or to obtain a larger response with the same stimulus. This form of secondary facilitation is prevented by severance of cortico-cortical connections, and hence they presumably play some rôle in it.

Strychninization of a few square millimeters within any subdivision of the central sector, performed under brief anesthesia from which the animal recovers before the effects of firing of the cortex have ceased, results in the appearance of all the familiar clinical signs of paresthesia and paralgesia in the apical portions of both sides of the body belonging to the subdivision strychninized (27). The reason for the bilaterality of the reference is by no means clear, for the afferent impulses are only contralateral from the arm and leg to the thalamic nuclei and to the cortex, and the symptoms are bilateral even when the strychnine is placed on an area practically devoid of callosal connections; so that neither cortico-thalamic nor cortico-cortical connections seem adequate to account for the bilaterality of reference implicit in the overt behavior. That these sensory symptoms are due to thalamic rather than to cortical excitation can be deduced from this. Strychninization of a few square millimeters of the most anterior portion of leg or arm subdivision fires all of both subdivisions, but only the thalamic nucleus of the subdivision strychninized and the symptoms are referred to the corresponding members. Moreover, strychninization of the thalamic nucleus in the cat is sufficient to produce the symptoms, even when the cortex has been removed. It was on the basis of these symptoms of sensory excitation that Dusser de Barenne outlined the "sensory cortex" and subdivided it according to the part of the body subserved—leg, arm or face. It was his hypothesis that the symptoms were to be explained by projection from every part of any subdivision to all parts of its thalamic nucleus. That hypothesis has been amply substantiated by recording the electrical activity of each thalamic nucleus during strychninization of each of the constituent areas of each subdivision. His "sensory cortex" consisted of all those areas which Brodmann numbered 1 through 7 in his map of the monkey's brain. Two difficulties have arisen with respect to this sector. The lower margin of the face subdivision has proved to be higher on the frontal and parietal operculum than was originally supposed, and the method of local strychninization and recording of resultant electrical activity, which he called physiological neuronography, has compelled distinctions between parts of the cortex histologically indistinguishable by present methods. To meet these difficulties and to indicate areas which have been identified cyto-architectonically or physiologically since Brodmann's maps, we made figure 2, a, b and c. (Hereafter numerals will be used to designate areas indicated by them

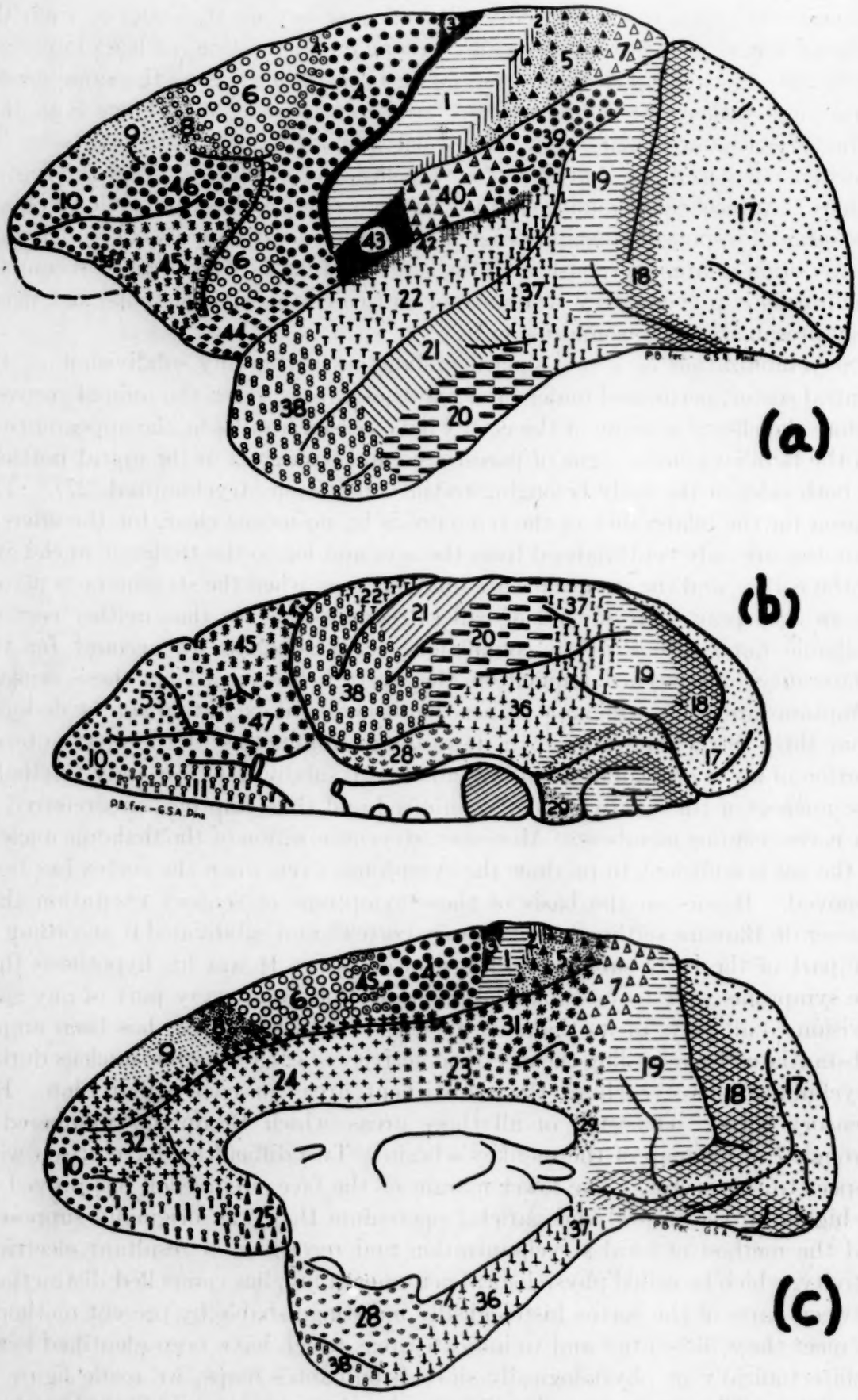

Fig. 2. *Macaca mulatta*. Areas of cortex distinguishable by cytoarchitectonics or physiological neuronography: (a) lateral, (b) orbital and (c) medial surface.

in this figure.) The central sector, or "sensory cortex," consists of the areas 6, 4s, 4, 3, 1, 2, 5. In the monkey, 7 and both 40 and 39 (of whose cytoarchitectural identity with area 7 Brodmann was himself uncertain) are all sensory in Dusser de Barenne's sense of the term, and both fire the postero-lateral thalamic nuclei but poorly. In the chimpanzee, 40 and 39 fail to fire it. In both primates they fire heavily into the pulvinar. Therefore, although their principal cortico-cortical connections are with the central sector, they must be considered as belonging to the parieto-temporal region, which is dominated by the sector of the pulvinar. Excluding these and the opercular areas, the firing from one to another of the component areas can be stated briefly; 6, leg or arm, fires all component areas of both subdivisions; 4 fires itself, 1, 2 and 5; only in the face subdivision does it fire face 6; 1, arm and leg, each fires itself locally, and the corresponding 4 and 5 well, whereas 1 face fires 6 face and 4 face; 5, arm and leg, each fires itself more widely, and the corresponding 4, 1, 2 and 7; 4s and 2 fire themselves locally and suppress the electrical activity of the cortex generally. They also suppress motor activity. To this region neither 44 nor 43 properly belong, although there is evidence that taste may be projected upon 43 (28) and although motion of the tongue and larynx are obtained from 44 by primary stimulation and from 43 by secondary facilitation.

SYLVIAN REGION. For descriptive purposes, 43 and 44, although firing face 6, are considered part of the sylvian region, which is dominated by projection from the medial geniculate body to a slight elevation, corresponding to Heschl's gyrus, running from near the middle of the lower lip of the sylvian fissure diagonally upward and inward. This corresponds to the primary auditory cortex of the cat, upon which the organ of Corti maps point for point. The arrangement is exactly what is to be expected when the twist of the region from cat to monkey is kept in mind (29). Lower tones are represented on its anterior or lateral end, and higher tones more posteriorly or medially. Still farther posteriorly, extending to the end of the sylvian fissure and sometimes emerging into the parietal region in positions corresponding to Campbell's "audito-psychic" area in the human brain, is what is probably best called the secondary auditory cortex, resembling the corresponding area in the cat. The "primary" area, 41, hidden in the sylvian fissure, is koniocortex, and the "secondary," 42, the para-acoustic area, contains very large pyramidal cells in layer iii. These areas are difficult to expose. Even subpial resection of the parietal operculum leaves them in a condition which makes truly local strychninization difficult. At present it seems best to confine remarks to the following statements: 41 fires 42; 42 fires itself well, and fires 41, as well as adjacent portions of 22. The posterior part of the superior temporal convolution fires area 42, but it is uncertain whether this is referable to strychninization of 22 or 37. No definite relation to any of the posterior parietal areas has been established, although in the cat partial asphyxia will cause a response to a click to appear in them synchronously with the response of the auditory cortex. The requisite opercular destruction prevents study of the connections of one to the other beneath the sylvian fissure, except from the surfaces exposed in the intact cortex. These have shown the following connec-

tions: 43 fires 44 and 22; 22 fires 44 and 43 and probably 42. Thus this region contains structures necessary for vocal response to auditory stimulation, and their cortico-cortical connections are direct.

PARIETO-TEMPORAL REGION. The region lying between the occipital posteriorly and the central and sylvian anteriorly, is roughly triangular with an extension forward on the medial aspect, whence it extends to a wide base along the inferior aspect of the temporal lobe. It includes 31 and probably 7 on the medial aspect, and 39, 40, 37, 20 on the convexity of the hemisphere. It is dominated by areas 39 and 37 which receive the bulk of the pulvinar projections. Each of its constituent areas fires itself well but its inter-areal connections are relatively few or restricted. 31 fires none of the other constituents. The line between 39 and 40 is vague, and these areas may fire each other, but only from neighboring portions. The upper limit of 37 is equally vague, and 37 fires 39. Similarly, more work is necessary to bound 37 anteriorly, but there is nothing to suggest that it fires or is fired by areas anterior to it. Per contra, its inter-regional relations are obvious and important, as will appear later.

LIMBIC REGIONS. The posterior limbic region, or sector of the anterior nucleus (30), lying between 31 and the corpus callosum, is made of two areas: one, which fires only itself and that only locally, called 29 for histological reasons; the other, 23, which fires itself throughout. The anterior limbic region is a single suppressor area, 24, which, like other suppressor areas, fires itself and only locally. Of areas near its anterior end—i.e., 25 and 11, we lack information and hence are unable as yet to assign them to this region or to the orbito-temporal or frontal regions.

FRONTAL REGION. The frontal region is bounded posteriorly by the central, medially by the anterior limbic and orbitally by 11, 47 and 53. In the monkey this region has not been clearly analyzable by local strychninization and distribution of firing within the region, except for two areas: 32, which fires itself throughout but no other frontal area, and 8, which is a suppressor area and fires itself extremely locally and fires into 32. Moreover, area 8 is "motor" for contralateral, and anterosuperiorly for ipsilateral, eye movements associated with dilatation of the pupil. This dilatation, in the case of the cat, is due to inhibition of the third nerve nucleus (31). As of eye movements obtained from 17, 18 and 19, it is possible to conclude that the cells of origin for the requisite structure lie in the area in question. Eye movements are obtainable from the parietal cortex generally, if short, high voltage pulses are used. These can stimulate underlying white matter, and thermocoagulation of the parietal cortex does not prevent them from being elicited, whereas it abolishes those evoked from 8. Unilateral destruction of 8 produces a pseudo-hemianopia (32) and circus movements. Immediately anterior to its central third lies 46, which receives the densest projection from the dorso-medial nucleus. Its strychninization results in relatively wide firing of the frontal pole. Anterior to it is 10 where firing is most restricted. The cortex medial, or dorsal, to area 46, being bounded by areas that can be thus defined, is called 9. The area ventral, or lateral, to 46—i.e., 45, is distinguished by its firing 44 and 40.

ORBITO-TEMPORAL REGION. The remainder of the isocortex, characterized by deficit of thalamic connections, constitutes the orbito-temporal region. The orbital areas, 47 and 53, are easily distinguishable, for electrical stimulation of 47, but not of 53, causes an arrest of respiration in inspiration with the vocal cords abducted—as in a yawn. Moreover, they fail to fire each other and are cyto-architecturally distinct, for 47 is dysgranular and 53 is eugranular. The temporal areas 38, 21 and 22 are difficult to distinguish histologically in the monkey. Each fires itself throughout but fails to fire the others. The relation of the orbital to the temporal components is as follows: 47 fires 38; 53 fires 37; 38 fires 47; 22 fires 53. It seems safe to assume that these connections form the bulk of the fasciculus uncinatus.

INTRACORTICAL CONNECTIONS. While we have been considering only the inter-cortical connections as revealed by strychninization under dial narcosis, it must be remembered that the belt of intra-cortical fibers exists and under other conditions can be shown to relate relatively distant foci. As these connections run in all directions, they relate areas regardless of the regions or sectors to which they belong. They cannot, however, relate an area to a distant one without crossing those areas which surround the excited area. This is strictly a topological problem in two dimensions. It is, therefore, invariant under continuous deformation of the cortex. Hence it is possible to map the entire cortex and the basal ganglia on a plane without losing the relations determined by the intracortical connections. Figure 3 is such a map. In it the margins of the cortex of one hemisphere appear stretched around the periphery, and the adjacent areas are consequently greatly elongated. Moreover, all the sulci are treated as if they were opened out. This, for example, discloses area 3 and the continuity of area 6 from arm to face subdivisions, and brings to the surface the insular cortex. In so doing it makes clearer the lack of information. The basal ganglia have been so displaced as to make them visible without severing their continuity with cortical structures either anteriorly, where 47 is continuous with the putamen, or posteriorly, where the amygdaloid nucleus adjoins the allocortex of the temporal lobe.

INTER-REGIONAL CONNECTIONS. The connections between the occipital and parieto-temporal regions probably constitute both the fasciculus longitudinalis inferior and the fasciculus occipitalis verticalis (of Wernicke), and the long antero-posterior connections, the superior longitudinal fasciculus. Just at the time that the connection of all suppressor areas to the belt-like areas 31, 32, were discovered by physiological neuronography, the connection of 4s to this same band was followed by myelin stain (33). Most of the remaining connections as yet lack correspondingly precise anatomical footing. Since the corpus striatum not only arises in parallel with the cerebral cortex, but also acts in parallel with it, at least in suppression of motor activity, the cortico-striatal connections established by physiological means are here included. Areas 24, 8, 4s, 2 and probably 19 all fire the nucleus caudatus, whereas 6 fires the putamen and the external segment of the globus pallidus, and 4 fires the putamen but not the external segment of the globus pallidus. Recently, by a new method applied

to the cat's brain, connections from some suppressor areas to the nucleus caudatus have been shown to be by fine nonmyelinated collaterals from descending axons (34). This accounts for previous failures to find them by Marchi studies of degeneration (35).

INTER-HEMISPHERICAL CONNECTIONS. For the most extensive anatomical studies of the connections of the two hemispheres we are indebted to Mettler (36, 37, 38, 39), according to whom almost all parts of one hemisphere send

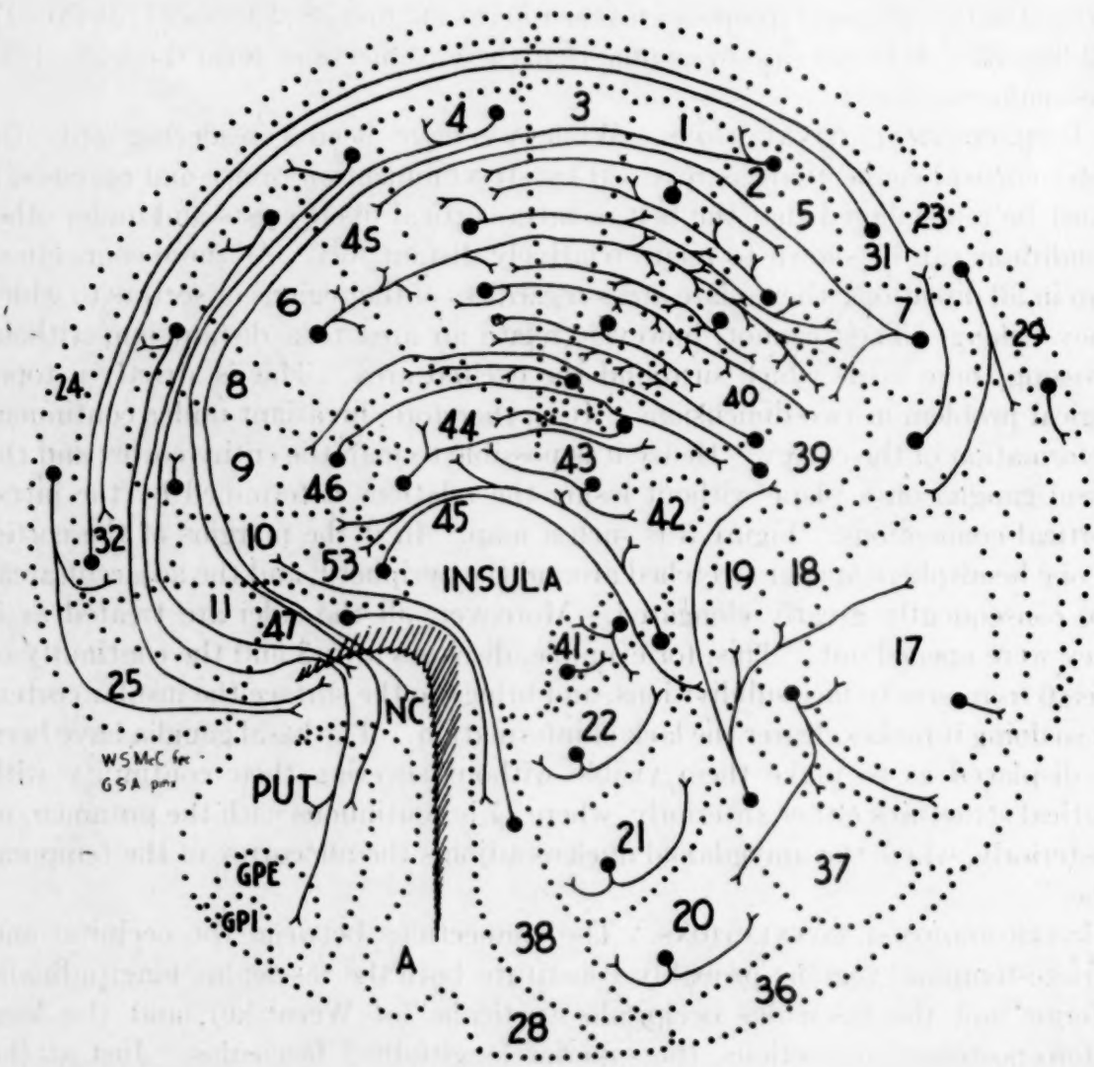

Fig. 3. *Macaca mulatta*. Development of cortex and basal ganglia preserving topological relations. On it are indicated all cortico-cortical and cortico-striatal connections demonstrated by physiological neuronography.

axons to a corresponding but larger and related portion of the other, while certain regions send them to many portions of the opposite hemisphere—so-called heterotopic connections. Possibly because the lesions affected the underlying white matter, the projections described by him are more extensive than those found by electrical stimulation, although this may have excited certain of the more superficial fibers. The reason for the difference may also be that the discovery of the electrical disturbance on the receiving hemisphere depends

necessarily upon the synchronous activity of enough axonal endings in a small space. A diffuse projection might easily be missed. This applies also to physiological neuronography, with this difference: that the strychnine can excite only neurons, not axons or axonal terminations. Hence its findings can only be of fewer projections than by these other means. Figure 4 schematizes the origin of the commissural connections as revealed by physiological neuronography. It discloses the direction as well as the most concentrated axonal distributions rather than their totality.

Mettler used the term "homoiotopic" for the more diffuse anatomical projections to corresponding related portions of the contralateral hemisphere. These are always restricted to constituent areas of the same contralateral region. Even what he has called "heterotopic" connections rarely exceed these limits.

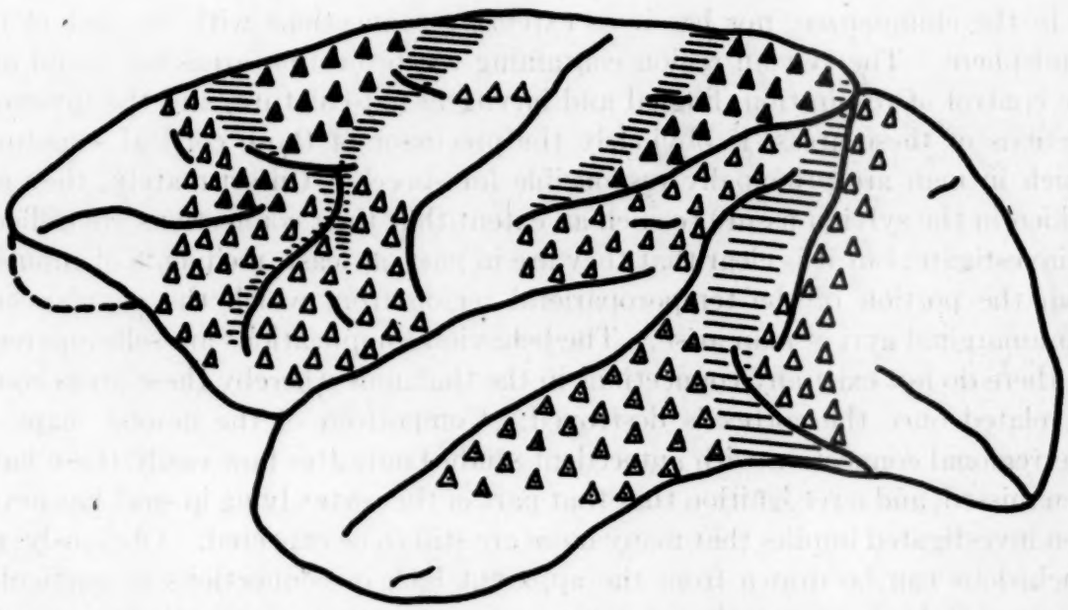

Fig. 4. *Macaca mulatta*. Cortical origins of commissural connections ▲, to widely separated points; △, to symmetrical point only and stopped by section of corpus callosum; ◬, to symmetrical point only but persisting though diminished after section of corpus callosum.

When they do so the exceptions conform to the interregional connections of the homolateral projections. There is an additional reason, therefore, for inquiring into the latter in some detail.

REMAINING DIFFICULTIES. In the first place, the study of the interregional connections has not been pushed as hard as the studies of the functional organization of the regions themselves, and there may be many connections as yet undiscovered, but the connections so far revealed are so numerous and intricate as to make any regional subdivision seem somewhat arbitrary. This is particularly true with respect to areas 31 and 32, which constitute a narrow band along the sulcus calloso-marginalis, with extensions at its anterior and posterior extremities. The extremities are clearly different, histologically, but in the intermediate region both areas shade into the adjacent areas of the central region so that no definite boundaries can be established. Moreover, this band is

internally connected by axons from any part to all parts in the monkey, whereas in the chimpanzee the connections are by no means as strong to distant portions. In the monkey, all parts of the band are fired from all suppressor areas, whereas in the chimpanzee the firing is more restricted. To assign different portions of this band to different regions seems arbitrary in spite of the dissimilarity of thalamic connections. A similar difficulty occurs with 7, 39 and 40, which are unmistakably sensory (as that term was used by Dusser de Barenne). Moreover, they have powerful connections to and from the rest of the central sector despite their projections to and from the pulvinar. The entire parieto-temporal region is an extreme example of long, interregional connections, for there is no region on the convexity of the hemisphere with which it fails to make connections in both directions. Inferentially, it subserves associational functions of a high order. The frontal region obviously has not differentiated as far in the monkey as in the chimpanzee, nor has it as extensive connections with the rest of the hemisphere. The sylvian region containing the projection areas for sound and the control of respiration, lingual and laryngeal musculature, and the interconnections of these areas, is obviously the precursor of those cortical structures which in man are principally responsible for speech. Unfortunately, they are hidden in the sylvian fissure to such an extent that their connections are difficult to investigate; but it is clear that they are in part, at least, recipients of impulses from the portion of the temporoparietal region from which the angular and supramarginal gyri of man arise. The behavioral implications are self-apparent, for there do not exist any connections in the thalamus whereby these areas could be related once the cortex is destroyed. Comparison of the newest maps of interregional connection with antecedent studies indicates how easily these have been missed, and a recognition that that part of the cortex lying in sulci has never been investigated implies that many more are still to be expected. Obviously, no conclusions can be drawn from the apparent lack of connections of particular regions. Only one general statement concerning these connections seems to hold: namely, that the primary sensory areas do not give rise to interregional associational fibers. This again emphasizes the importance of the "associational areas." It is apparent today that certain tenets of the psychology of perception can be deduced from the physiology of the primary sensory areas. In similar fashion we have every right to expect that utimately knowledge of "associational areas" and of interregional connections will lead to a physiological interpretation of the more complex psychological problems.

In order to bring this article (which is essentially a review) as nearly up to date as possible, I have taken the liberty of including hitherto unpublished work done in collaboration with G. von Bonin, H. W. Garol, E. W. Davis, A. Silveira and P. Bailey—to the last of whom we are indebted for figure 2.

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